ANTI-CANCER ACTIVIY OF NAMACHIVAYA CHENDOORAM IN IN-VITRO CELL LINE MODELS AGAINST INVASIVE CERVICAL CARCINOMA

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Anti-Cancer Activity of *Namachivaya Chendooram* in In-vitro Cell Line Models against Invasive Cervical Carcinoma"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.Velpandian M.D(S), Ph.D.,** Post Graduate Department of *Gunapadam*, Govt.Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ABBREVIATIONS

ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Amino Transferase
ANOVA	Analysis of Variation
BUN	Blood Urea Nitrogen
CT	Computed Tomography
COX	Cyclooxygenase
CMC	Carboxy Methyl Cellulose
CAMP	Cyclic Adenosine Monophosphate
CPCSEA	Committee for the Purpose of Control and Supervision of Experimental Animals.
DMEM	Dulbecco's Modified Eagle's Medium
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DNA	DeoxyRibo Nucleic acid
DC	Differential Count
DSC	Differential Scanning Calorimeter
EDX	Energy Dispersive X-ray Spectrometry
FDG-PET	F-18 Fluoro-2-deoxy-D-glucose

FAD-Assay	Flavine Adenine Dinucleotide
FTIR	Fourier Transform Infrared Spectrometry
GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
HPV	Human Papilloma Virus
HSV2	Herpes Simplex Vrus type-2
HDL	High Density Lipoprotien
ICPOES	Inductively Coupled Plasma Optical Emission Spectrometry
IAEC	Institutional Animal Ethical Committee
ICMR	Indian Council of Medical Research
LDL	Low Density Lipoprotein
LD50	Lethal Dose
MCV	Mean Corpuscular Volume
MRI	Magnetic Resonance Imaging
MTT	3-(4, 5-Dimethylthiazol-2-yl)-2, 5- Diphenyl Tetrazolium Bromide
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
NCRP	National Cancer Registry Programme
OECD	Organisation for Economic Corporation and Development
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume

PET	Positron Emission Tomography
RBC	Red Blood Cells
SEM	Scanning Electron Microscope
SEM	Standard Error Mean
SGOT	Serum Glutamate Oxaloacetate
SGPT	Serum Glutamate Pyruvic Transaminase
VLDL	Very Low density Lipoprotein
WDS	Wavelength Dispersive Spectroscopy
WBC	White Blood Corpuscles
WHO	World Health Organization
XRD	X-Ray Diffraction

1. INTRODUCTION

The Siddha Medical Science is one of the ancient Indian traditional medicine nearly followed by the Tamil speaking people, in India, Malaysia, Singapore, etc.,

All the systems of medicine mainly focus on the prevention and cure. Whereas Siddha system of medicine not only focus on prevention and cure but also emphasise in *kaya kalpa* i.e. making one's body immortal [1].

One of the well known Tamil poets *Tholkaapiyar* who introduced the Tamil grammar to the world refers the Siddhar as ***Arivars*** in his book "*Purathinaiyiyal*.

'kWtpy nrajp %t iff fhyKk
newpapd Mwwpa mwptH" - njhyfhggpaH

Nachinarkiniyar states that Arivar means "one who does not possess desire, hatred and illusion and remains unchanged in attitude in all situations (past, present and future).

Not only our Tamil poets explained about the uniqueness of the Siddhar but also English philosopher like Sinclair Stevenson has fully praised Siddhar about their capabilities.

The term *Siddhi* refers to a Yogic state, Siddhar are said to be the Yogis, having lived a complete life [2].

Siddhar who sacrificed their entire life for the Siddha medicine has given miraculous remedy for incurable, chronic and non communicable diseases thousands of years back. Gyneacological diseases with treatment are mentioned in Siddha text.

In our ancient literature *Tholkaapiyam, Poruliyal* version, women are mentioned as follows,

'nrwpTk epiwTk nrkikAk nrgGk
mwpTk mUikAk ng z ghyhd" - njhyfhggpak

The above mentioned quotes denotes

“Those who are Intense and galore”

Who said to be Elegance?

Who is the knowledge of wisdom?

Who are of great beauty?

They were refined as women in the period of *Tholkaapiyar* [3].

Women suffered many chronic diseases from ancient times. Some of them are Maladu (infertility), Yoni Thabitha Noikal (pelvic inflammatory diseases), Marbu silanthi (breast cancer,) Vippurithi (tumour), Yoniputru (cervical cancer) etc.

Now from the above mentioned diseases one of the life threatening disorder which reduces the quality of life for a woman Yoni putru correlates with cervical cancer in modern world which considered to be the second most cause of death in women in India.

Cervical cancer is cancer that starts in the cervix, the lower narrow part of the uterus. It happens when the body’s cervical cells divide very fast and grow out of control. These extra cells form a tumour. Most of the cervical cancers are caused by the Human Papilloma Virus (HPV) [4].

PREVALENCE:

According to National Cancer Registry Program recent report of 2008, the load of breast and cervical cancer together was 23.6 to 38.7% of the total cancers [5].

In 2009 the number of cervical cancer cases were 1, 01,938 which has increased to 1, 07,690 in 2012. Among this Tamilnadu reported 55,000 new cases per year state wide in 2012 to 2016 [5a]. Day by day the morbidity is increasing.

Recent data:

New cases registered – 123000 /year

Deaths – 67500 /year

Median age – 38 years (age 21-67 years) [6].

Rural women are at higher risk of developing cervical cancer as compared to their urban counter parts. Cervical cancer is the second largest cause of cancer mortality in India accounting for nearly 10% of all cancer related deaths in the country.

It is estimated that by the year 2020 there will be almost 20 million new cases[7].

In our country where the risk factors of cancer cervix are prevailing such as early marriage, early conception, multi parity and low socio-economic condition.

Cervical cancer reflects the striking global health inequity, resulting in deaths of women in their most productive years, with a devastating effect on the society at large.

January the awareness month of cervical cancer

Even though the cervical cancer is acknowledged and the awareness is spread in the very beginning of the year, we are still running behind the solution till the last month of each and every year. Pap smear screening is vital to arrest the cervical cancer in the preliminary stages and it is very important that people should be exposed to this knowledge [8].

For this life threatening disease radiation is combined with low dose chemotherapy however this modality often leads to severe toxicity.

The well known practice of chemotherapy to reduce the risk factor of cancer also may leads to many adverse effects such as nausea, vomiting, alopecia (loss of hair), bone marrow depression, amenorrhoea in women like major problems [9]. So the failure of conventional chemotherapy to reduce mortality invites attention towards new alternative approaches that would reduce morbidity as well as side effects conferred by conventional chemotherapy.

Recently, a greater emphasis has been given towards the involving traditional Siddha medicine that includes herbal, Herbo -mineral and metallic preparations has been used from the immemorial to treat chronic ailments such as cancer. Siddhar are well known practitioners in preparation of a Herbo-mineral formulation by proper purification, using herbal juices to reduce the toxicity of the metals. A Siddha text

clearly specifies use of Mercury, Sulphur, Copper, Arsenic and Gold as therapeutic agents [10].

Some of the drugs that have already used in the treatment of cancer such as *Rasagandhimezhugu, Gowri chinthamani* [11], *Panchpadana Chendooram* by proper validation scientifically which already mentioned in Siddha literature as theoretical text. Siddha system of medicine believes that Herbo-mineral formulation to be more effective for chronic diseases [12]. Herbo-mineral formulations are gaining popularity worldwide due to its availability in the form of Nanoparticles, increased bioavailability, minimal side effect, longer shelf life period and need less dosage as compare to herbal formulations. Up to date, lesser studies have been conducted on standardization of such preparations [13].

On this pathway the trial drug *Namachivaya Chendooram* may reach the next higher level in treating cervical cancer by the upcoming activity on pharmacological, toxicological and analytical studies.

Why Nanomedicine a new search to treat chronic ailments especially cancer?

Nanomedicine are used globally to improve the treatments and lives of patients suffering from a range of disorders including cervical, ovarian and breast cancer, kidney disease, fungal infections, elevated cholesterol, menopausal symptoms, multiple sclerosis, chronic pain, asthma and emphysema.

The Nanomedicine that is currently available is overcoming some of the difficulties experienced by normal medical approaches in delivering the benefit from the drug molecules used. Drugs may lead to side-effects due to poor delivery at the actual site of disease. For example, drugs that are targeting cancers must avoid healthy tissues and organs or damage can be caused. Nanomedicine therefore can play an important role in ensuring enough of the drug enters the body, that drug that does enter stays in the body for long periods and is targeted specifically to the areas that need treatment [14].

Over the coming years, the benefits of Nanomedicine and new diagnostic tools will be felt by an increasing number of patients with considerable impact on global health.

An integrated approach is the need of the day to manage cancer using the growing knowledge gained through scientific development. The emerging integrative nanomedicine model of cancer treatment recognizes the importance of herbo-mineral medicine. The Author is interested in proving the trial drug *Namachivaya Chendooram* which is literally evident as told by great scientists Siddhar. The need of the hour is to develop a platform for scientific validation which may satisfy the cost effective and affordable treatment for cervical cancer.

2. AIM AND OBJECTIVES

AIM

The aim of this study was to validate the safety and efficacy of the herbo-mineral formulation of ***Namachivaya Chendooram*** for its anti-cancer activity in in-vitro cell line models.

OBJECTIVES

Objectives are surfaced in the below mentioned points,

- Collection of relevant literature from Siddha and modern text.
- Standardization of the preparation of drug according to classical Siddha text.
- Evaluation of physicochemical analysis
- Biochemical analysis for determining acidic and basic radicals.
- Estimation of elements through instrumental analysis.
- Evaluation of acute and sub-acute toxicity studies according to OECD guidelines.

Facts to be proved by pharmacological activities include

- Anti-cancer activity in HeLa cell line models by MTT assay,
- Anti-tumour activity in SiHa cell line models,
- Anti-oxidant activity by DPPH assay.

3. REVIEW OF LITERATURE

3.1DRUG REVIEW

3.1.1.SIDDHA ASPECT

SIDDHA ASPECT OF *RASAM*

Chemical name: *Rasam* (Hydragyrum) (Mercury or Quick silver)

Mercurycomes under the classification of '*Panchasoothaam'*. It has many connotations such has *sootham, punniyam, bharatham, inimai*, *sivasathi, kesari*etc, according to*Dasanganigandu.*

Mercury is obtained from its ores in countries like Spain, California, Russia, China and Japan. It is separated from its ore Cinnabar.

Antagonists to Mercury:

Singi, Gowri, Vellai, Kudhirai pall, Saththicharam.

Agonists to Mercury:

Appragam, Kaareeyam, Silai, Kenthi, Veeram.

Types of Mercury:

Mercury was classified into five types.

1. *Rasam*
2. *Rasendhiran*
3. *Sootham*
4. *Misaragam*
5. *Baaratham*

Properties:

1. Vitalizer
2. Tonic

3. Laxative
4. Diuretic
5. Neutralising *pitham*
6. Sialagogue
7. Anti inflammatory
8. Medicine for venereal disease

Taste: Six tastes dominated by sweet.

Potency: Hot and cool (both -speciality)

Special properties of Mercury:

Unlike other drugs Mercury is useful in the treatment of diseases caused by both heat and cold.

***Dhosam* (Impurities) of Mercury:**

It is considered that there are two types of *dhosam* of Mercury. They are

1. Dhosam

2. Sattai (Kavasam)

In *Dhosam* there are 8 types of impurities in Mercury producing various diseases as shown below

Impurities Disease caused by them

1. Undheenam Soolai (Throbbing pain)

2. Kowdilayam Kapalanoi (Disease of the head)

3. *Anavartham Biramai* (Manic illness)

4. *Sangaram Thathunattum* (Spermatorrhoea)

5. *Sandathvam* Distress

6.*Panguthvam Kuttam* (Leprosy)

7. *Samalathvam Moorchai* (Syncope)

8. *Savisthavam Sareera Elaippu* (Loss of weight)

Sattai is an another one classification, there are 7 types of impurities in Mercury which producing various diseases as shown below

ImpuritiesDisease Caused by Them

1. *Naagam Moolam* (Haemorrhoids)

2. *Vangam Tholnoikal* (Skin disease)

3. *Malam Arivinmai* (Idiocy)

4. *Vidam Maranam* (Death)

5. *Akkini* Morbid thrist (Polydypsia)

6. *Giri Sattium* (Distress)

7. *Sabalam Thathunattam* (Spermatorrhoea)

General properties of Mercury:

‘ t‰oNeha f‰uej‰F dkk nka# i y G z F l

lo‰fhy‰y t‰eJ t‰dhy mj i j-to‰aha

Gh‰a t‰j‰ahJ Gh‰aNdh nayyhk

,h‰A t‰j‰ahJ k‰y i y”.

- F z ghlk jhJ r t tFgG

Proper use of Mercury as a medicine can able to cures the following diseases they are disease in eyes, syphilis, eight types of ulcers (*gunmam*), throbbing pain (*soolai*), chronic ulcers (*perumpun*) and Leprosy (*kuttam*) [15].

Purification and detoxification of Mercury:

- Mercury - 35gram
- Brick powder - 100gm
- Turmeric powder - 100gm
- *Acalypa indica* juice - 1.3 lit

Mercury is triturated with brick powder and then turmeric powder for one hour respectively and washed with water cleanly. Then the Mercury is boiled with the juice of *Acalypha indica*, it is detoxified and then finally it is washed with water then stirs it by using cotton cloth Mercury is purified [16].

Preparations of Mercury:

- *Sootha karuppu*
- *Rasa mezhugu*
- *Rasa thailam*
- *Megavirana kalimbu*
- *Rasa kuligai*

3.1.2. MODERN ASPECT

MODERN ASPECT OF MERCURY

Mercury is the only common metal which is liquid at ordinary temperatures. Mercury is sometimes called quicksilver. It is a heavy, silvery-white liquid metal. It is a rather poor conductor of heat when compared with other metals but it is a fair conductor of electricity. It alloys easily with many metals, such as Gold, Silver, and Tin. These alloys are called amalgams.

CHEMICAL PROPERTIES OF MERCURY

Atomic number	-	80
Atomic mass	-	$200.59 g.mol^{-1}$
Electro negativity	-	1.9
Density	-	$13.6 g.cm^{-3}$ at $20^{\circ}c$
Melting point	-	$-38.9^{o}c$
Boiling point	-	$356.6^{\circ}c$
Radius	-	0.157nm
Ionic radius	-	0.11nm (+2)
Isotopes	-	12
Electronic shell	-	$[Xe]4f^{14}$
Standard potential	-	+0.854V

MERCURY SALTS:

The most important Mercury salts are mercuric chloride $HgCl_2$ (corrosive sublimate - a violent poison), mercuric chloride Hg_2Cl_2 (calomel, still used in medicine occasionally), Mercury fulminate (Hg $(ONC)_2$, a detonator used in explosives) and mercuric sulphide (HgS, vermillion, a high-grade paint pigment)

Applications [17]

- Mercury metal has many uses. Because of its high density it is used in barometers and manometers. It is extensively used in thermometers, thanks to its high rate of thermal expansion that is fairly constant over a wide temperature range. Its ease in amalgamating with gold is used in the recovery of gold from its ores.
- Industry uses Mercury metal as a liquid electrode in the manufacture of chlorine and sodium hydroxide by electrolysis of brine. Mercury is still used in some electrical gear, such as switches and rectifiers, which need to be reliable, and for industrial catalysis. Much less Mercury is

now used in consumer batteries and fluorescent lighting, but it has not been entirely eliminated.

- Mercury compounds have many uses. Calomel (Mercurous chloride, Hg_2Cl_2) is used as a standard in electrochemical measurements and in medicine as a purgative. Mercuric chloride (corrosive sublimate, $HgCl_2$) is used as an insecticide, in rat poison, and as a disinfectant. Mercuric oxide is used in skin ointments. Mercuric sulphate is used as a catalyst in organic chemistry. Vermilion, a red pigment, is mercuric sulphide; another crystalline form of the sulphide (also used as a pigment) is black. Mercury fulminate $Hg(CNO)_2$ is used as a detonator.

Mercurial preparations:

- Mercury with Chalk (Grew powder)
- Yellow mercuric oxide (HgO)
- Mercuric oxide
- Oleated Mercury
- Mercurous chloride (HgCl-Calomel)

Tests for Purity:

It has been tested for weight per ml (at 25°C is about 13.5g).Non volatile matter residue at 300°C (not more than 0.02%w/w)

Assay:

An acuity weighted quantity (0.49g) is dissolved in equal parts (20ml) of water and nitric acid. It is heated gently until the solution become colourless. The solution is then diluted with water (150ml) and sufficiency quantity of potassium permanganate is added till a permanent pink colour is produced. A trace of ferrous sulphate to discharge pink colour is added. Then the solution is titrated with standard 0.1N Ammonium thiocyanate (1ml of 0.1N Ammonium thiocyanate =0.01003g), using ferric ammonium sulphate as indicator. The temperature during the titration should not exceed above 20°C.

$3Hg+8HNO_3 \rightarrow 3Hg(NO_8)2+4H_20+2NO\uparrow$

$Hg(NO_3)2+2NH_4SCN \rightarrow 2NH_4NO_3+Hg(SCN)\ 2$

Uses:

It finds as an pharmaceutical aid and for preparing Mercury with chalk .Formerly metallic Mercury found use as such therapeutically as a cathartic and parasiticide .But it is more used as such, as it has been extremely poisonous and prolonged inhalation of even very minimal amounts of Mercury prove fatal. Almost all the salts of Mercury with the exception of the sulphide, has been poisonous.

1. Mercury with chalk (Grew powder)

- It is having 31 -35% w/w of Mercury and 62-70% w/w of $CaCO_3$
- It is used as a purgative (Dose 60-300mg)

2. Yellow mercuric oxide (HgO)

- It is having not less than 99.5% HgO
- It is used as a mild antiseptic and used as anti infective and anti bacterial agents.

3. Mercuric Oxide:

- It contains not less than 95% but not more than105% w/w of the stated amount of yellow mercuric oxide
- It is used in ophthalmology, 1% ointment to treat mild inflammatory conditions for the treatment of blepharitis and conjunctivitis.

4. Oleated Mercury:

- It has the equivalent of 20% of yellow mercuric oxide
- It is used as an anti infective.

5. Mercuric chloride (HgCl) (Calomel):

- It is being not less than 99.6% of HgCl

- It has been used for centuries as a cathartic but recently it is replaced by other drugs.
- Calomel has been insoluble in gastric juice and has been not absorbed from the stomach. It gets absorbed in the intestine by the alkaline pancreatic juice where it slowly gets dissociated into Mercury and irritant mercuric compounds which have been exerting a cathartic action

1. SIDDHA ASPECT OF *Thaalagam*

English names:

Yellow arsenic trisulphide, trisulphuret of arsenic, orpiment.

Other names:

Peethagi, Aalmbi, Paluppu, Kothantham, Maalam, Arithaaram, Kaalpuththi, Ponvarni, Manjalvarni, Maaldevi and Arithalam.

Types:

Depending upon the colour, appearance and properties, *Thaalagam*has beenclassified into four types.

1. *Sivappu Aridharam* (Red Orpiment)
2. *Madal Aridharam*
3. *Pon Aridharam* (Gold Orpiment)
4. *Karattu Thalagam*

General properties: [18]

‘jhsfjjpd NgUiuff jhYfTs NehaF\lk

esf Fspfharry eLfgk-ehshfqnfhs

J\lggwqfpgGz #oOfz kziI Neha

fplIggLkh fpsjJ”

-gjhHjj Fz rpejhkzp

It is effective in the treatment of skin diseases, disease of head and tongue, fever with chills, *kapha* diseases, urinary tract diseases, and venereal focus ulcer in the urethra.

Actions:

Expectorant, antipyretic, convalescent, tonic and emetic.

Other medicines:

Thalagaennai (Virana sanjeevi thailam) – Healing of chronic ulcers.

Signs and symptoms of yellow arsenic poisoning:

Yellow arsenic if not prepared properly, the preparation will be toxic. The following symptoms are seen. Burning pain of the stomach, gastritis, hoarseness of voice, nasal bleeding, bleeding from the nail buds, itching over the head, redness in the tip of the hairs, mental disorders, lower abdominal swelling, and throbbing pain in the back, bronchitis and sciatica.

Antidote:

Root bark of Ceylon lead wort (*Plumbago zeylanica*)	_	8.75gm
Pepper (*Piper nigrum*)	–	8.75gm

These are added together and made decoction. Culinary salt (4.37gm) is then added and the mixture is taken twice daily for 21 to 42 days.

MODERN ASPECT OF ARSENIC TRISULPHIDE

Arsenic compounds have been known since at least the days of Ancient Greece and Rome (thousands of years ago). They were used by physicians. The compound most often used for both purposes was arsenic sulphide (As_2S_3) [19].

Arsenic was first recognized as an element by alchemists. Alchemy was a kind of pre-science that existed from about 500 B.C. to about the end of the 16th century.

PROPERTIES:

SYMBOL	-	As
ATOMIC NUMBER	-	33
ATOMIC MASS	-	74.9216
FAMILY	-	Group (VA)
PRONUNCIATION	-	AR-se-nick

A small amount of arsenic is used in alloys. An alloy is made by melting and then mixing two or more metals. The mixture has properties different from those of individual metals. The most important use of arsenic in the United States is in wood preservatives.

Physical properties

Arsenic occurs in two allotropic forms. Allotropes are forms of an element with different physical and chemical properties. The more common form of arsenic is a shiny, gray, brittle, metallic-looking solid. The less common form is a yellow crystalline solid. It is produced when vapours of arsenic are cooled suddenly.

When heated, arsenic does not melt, as most solids do. Instead, it changes directly into a vapour (gas). This process is known as sublimation. However, under high pressure, arsenic can be forced to melt at about 814°C (1, 500°F). Arsenic has a density of 5.72 grams per cubic centimeter.

Chemical properties:

Arsenic is a metalloid. A metalloid is an element that has properties of both metals and non-metals. Metalloids occur in the periodic table on either side of the staircase line that starts between boron and aluminium.

When heated in air, arsenic combines with oxygen to form arsenic oxide. A blue flame is produced, and arsenic oxide can be identified by its distinctive garlic-like odour.

Arsenic combines with Oxygen more slowly at room temperature. The thin coating of arsenic oxide that forms on the element prevents it from reacting further.

Arsenic does not dissolve in water or most cold acids. It does react with some hot acids to form arsenous acid or arsenic acid (H_3AsO_4).

Occurrence in nature:

It is usually found as a compound. The most common ores of arsenic are arsenopyrite, orpiment (As_2S_3), and Realgar (As_4S_4). These compounds are obtained as a by-product of the mining and purification of Silver metal.

The abundance of arsenic in the Earth's crust is thought to be about 5 parts per million. That place the bottom third of the elements in abundance in the Earth's crust.

The world's largest producers of arsenic are China, Chile, Mexico, Belgium, Namibia, and the Philippines. The United States does not produce any arsenic.

Isotopes

One naturally occurring isotope of arsenic exists, arsenic-75. Isotopes are two or more forms of an element. Isotopes differ from each other according to their mass number. The number written to the right of the element's name is the mass number. The mass number represents the number of protons plus neutrons in the nucleus of an atom of the element. The number of protons determines the element, but the number of neutrons in the atom of any one element can vary. Each variation is an isotope.

A radioactive isotope is one that breaks apart and gives off some form of radiation. Radioactive isotopes are produced when very small particles are fired at atoms. These particles stick in the atoms and make them radioactive. About 14 radio isotopes of arsenic are available.

None of the isotopes of arsenic have any important commercial use.

Extraction:

The process of recovering arsenic from its ores is a common one used with metals. The ore is first roasted (heated in air) to chemically convert arsenic sulphideto arsenic oxide. The arsenic oxide is then heated with charcoal (pure carbon). The carbon reacts with the oxygen in arsenic oxide, leaving behind pure arsenic.

2. SIDDHA ASPECT OF *KAANTHAM*:

English name – Magnetic oxide of Iron

Other names:

Sivaloga sevagan, Tharanikku naatham, Sootha angusam, Navaloga thurati, Kaaya chithikku paathiravan, Murugan puranam.

Types:

There are five varieties of *Kaantham*

1. *Kal kaantham (Piramugam)*
2. *Oosi kaantham (Kambagam)*
3. *Pachai kaantham (Karshagam)*
4. *Arakku kaantham (Dhiravagam)*
5. *Mayir kaantham (Romagam)*

General properties:

‘fhejjjhwNrhigFdkq fhkpy Nkfkghz L
NrHej jphpNjhl ntliil rjqfhy - Xaejgrp
NgUjuq fzNzhagpukpa euhikAkNghk
Xhpdpiwah A+SWk cd”.

-gjhHjj Fz rpejhkzp

In general, the *Kaantham* has got the similar properties as iron. However, it is considered that *Kaantham* is superior to iron in many aspects. This is very effective in the treatment of swelling, ulcer, jaundice, venereal diseases, *kaphavatha* diseases, leucorrhoea, dyspepsia, gonorrhoea, anasarca, eye diseases and splenomegaly. It also increases the life span [20].

Method of purification:

Magnetic oxide of iron- 35 gm

Root bark juice of *Ponnavarai* (*Cassia auriculata)*- 210 gm

Magnetic oxide of iron is soaked in root bark juice of *Ponnavarai* and isolated from morning to evening for ten days. Then it is dried for two days without adding the juice. This process is repeated twice and washed to obtain purified magnetic oxide of iron.

MODERN ASPECT OF MAGNETIC OXIDE

Chemical Name: Magnetic oxide

Synonyms:

Magnetite / Black Iron Oxide (Fe_30_4), super paramagnetic iron oxide , black iron sand, magnetite sand, beach magnetite sand, iron oxide (Fe_3O_4), magnetic black, magnetic iron ore, ferrous ferric oxide , magnetic oxide, , tritontetra oxide, ferrous ferric oxide, iron black, black Iron BM, iron (III) oxide, Meramec M 25, river sand, black gold F 89.

Magnetic Oxide Formula:

Fe_3O_4

Magnetic Oxide (Fe_30_4) Description:

a) Magnetite was a natural occurring iron oxide magnet, consequently the name giving its distinguishing characteristic.

b) Magnetite was a member of spinal group which has the standard formula A (B) 2O4. The A and B of this represent different metal ions that occupied in specific sites on its crystalline structure. In magnetite standard formula was Fe_3O_4, in this a metal represent Fe +2 and the B metal represent Fe +3; two different metal ions in two specific sites. This arrangement causes a transfer of electron ions between the different irons in a structured path or vector. This electric vector was responsible for generating a magnetic field.

c) Lustrous black, magnetic mineral occurs on crystals of the cubic system in masses, and as loose sand. It was one of the main ores of iron (magnetic iron ore) and is a common constituent of igneous and metamorphic rocks. It was found in various parts of the United States, Norway, Sweden, and the Urals. A variety of magnetite was lodestone or loadstone exhibits its polarity especially interesting for its natural magnetism.

d) Magnetite is sometimes found in large quantities in beach sand. Such mineral iron sands or black sands are found in various places of California and New Zealand west

coast lands. The magnetite was carried out from beach to rivers from erosion and it's concentrated via wave action and currents.

Chemical Properties:

- Purity Available: From 96 percent to 99.9 percent
- Super paramagnetic iron oxide was available in 10 microns size and had no magnetic memory.

Physical Properties:

- Lumps, pieces, targets, granules and various powder or particle granulations down to as small as 15 to 20 nano meters.
- Black iron oxide nano particles are presently available as smaller size from 15 to 20nanometers.

Nominal Physical Constants:

- Magnetite lustre: Metallic
- Magnetite tenacity: Brittle
- Magnetite ID Mark: Ferromagnetic
- Magnetite Solid Density (gm/cm3): 5.1
- Magnetite pH: 7
- Magnetite Transparency: Opaque
- Magnetite Hardness 20℃: ~ 5.5 to 6.5
- Magnetite Specific Gravity: ~ 5.17 to 5.18
- Magnetite Colour: Black to greyish
- Magnetite Crystal System: Isometric
- Magnetite Particle Shape: Irregular
- Magnetite Magnetic Properties: Ferric magnetic

Magnetite (Fe_3O_4) Typical Applications:

- Magnetite was a main ore form of iron. It is used mainly in various fields. Magnetite was used as a pigment for polishing compounds, cosmetics,

medicines, polymer & rubber filler, building & construction, appliances, and magnetic inks.

3. SIDDHA ASPECT OF *LINGAM:*

OTHER NAMES IN SIDDHA

1. Inkuligam, Rasam, Kadaivanni, Karppam, Kalikkam, Kaanjanam, Kaaranam, Saaniyam, Chendooram, Maniragam, Milecham, Vani and Vanni.

2. 'k z ÿthhÿ , qFyÿfk t d rhjÿ yÿqfk
   j zÿthUk fhHF z k rhUk-gÿ zÿkhiy
   jhoej gtÿFoyhaj jhqfhj jÿhÿNjhlk
   toejhdWk vdNw tÿskG”

   - mfjjÿaH itjjÿa rÿejhkzÿ

   - (ghly-2173)

3. 'kiythhÿ> kiyuhrk› kzÿehfk”

   - mDNghf itjjÿa etejk ghfk 4

4. 'tddÿaÿd nfHgg kfjjhd cz
   fddÿa ngUkhd fhuzkhk yÿqfk
   jddÿr rkurk rhHthd nreJ}uk
   mddÿg gÿwejÿLk yÿqfjjÿd ngaNu”

   - rllKdÿeÿfzL 1

-சட்டமுனி நிகண்டு 1

Action: Tonic

General character

‘NgjÿRuQ reeÿngU tÿuz enuhLj
fhjfb fhrqfug ghdGz-Nzhj
TUtÿyÿqf rqfjkhA+W flbAk Nghq
FUtÿyÿqf rqfkjijf nfhs”

‘M j‰a‰ uj T f fhj yhw rhj‰y‰q f

Nkhj‰y‰ uj F z KwW}l y‰w - wJGh‰

F l l q f‰uej‰n fhL Q # i y thj K j

Y l l q F Neha f i s Nah l L k”

- F z ghl k jhJ r t t FgG

This preparation is effective in the treatment of diarrhoea, pyrexia, delirium, utricaria, diuresis, tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain, (*soolai)* and *vatha* diseases.

Purification:

- Alangium bark (*Alangium salvifolium*) 1400 gm is powdered and added with vinegar 5.2ltrs and placed in dew in the night. The next day it is rubbed and kindled well.35grms of cinnabar is tied well in a cloth and put it into the above liquid. The pot is covered with another pot sealed with mud pasted cloth, dried and exposed in dew for one day.It is heated with low intensity fire (flame) until the liquid is dehydrated for 24hours.

  Then the cinnabar is taken out and cleaned well. This procedure is repeated using the vinegar soaked individually with the whole plant of *Vitis lanata* (*Pulikarunai*) and Indian Sarsaparilla root.

- Lime juice, cow’s milk and the Indian *Acalypha* juice are mixed in equal proportion and allowed to fuse cinnabar so as to get it in a consolidated potency state.

- When the crude form of red sulphide of Mercury is soaked for one day in mother’s milk and lemon juice respectively, it becomes purified.

- Cinnabar is soaked in mother’s milk for 30 *naazhigai* (72 minutes).It is removed and again fresh milk is added and the process is repeated above for 2 times.

Toxic symptoms of Lingam

- Loss of taste, difficulty in eating and drinking water.

- Ulcers in the buccal floor, uvula (base of the mouth) inner portion of the tongue, larynx and large intestine,
- Foul odour from the mouth, discharge of viscous, whitish saliva,
- Difficult to speak and burning sensation are the toxic features of red sulphide of Mercury.

Antidote

- Nutmeg (*Myristica fragrans*),
- Cubeb pepper (*Piper cubeba*),
- Root bark of red cotton tree (*Gossypium arboreum*)
- Sugar

All the above ingredients were taken equal quantity of 4.2gm are made into a decoction and administered twice a day for 48 days.

MODERN ASPECT OF CINNABAR:

Cinnabarite, Vermillion, Vermilion, Cinnared.

Vernacular Names:

Tamil	-	Elingam
Sanskrit	-	Lingam
Telugu	-	Inglieekam
Kannada	-	Chayilyam
Hindi	-	Hingool

Introduction

Cinnabar is the chief mineral composed of the element of Mercury and is very important ore of Mercury. It is a colourful mineral that adds a unique colour to the mineral. The term is also used to describe the bright red colour of this element.

Occurrence: It occurs in many parts of world, particularly in California, China, Spain, and Italy & United States.

Table: 1 General Properties

Physical properties	
Colour	Bright scarlet red or cinnamon red to brick red
Lustre	Adamantine to sub metallic in darker specimens
Transparency	Crystals are translucent to transparent
Crystal system	Hexagonal
Hardness	2 to 2.5
Specific gravity	8 to 8.1
Associated minerals	Pyrite, quartz, Mercury and Dolomite, calcite.
Chemical properties	
Chemical formula	HgS
Composition	Mercury (II) sulphide
	1. 86.22% - Mercury (Hg)
	2. 13.78%- Sulphide (S)

HgS which has long been used in combination with traditional Siddha and Chinese medicine as a Sedative, Hypotonic, Ant inflammatory, Anti pyretic and Analgesic for more than 2000 years and is still widely used in Asian countries [20].

The estimated human therapeutic dose of cinnabar in traditional medicine used to approximately 5 -25 mg /kg /day /per dose three times / day as indicated in pharmacopoeia of China (2000).

An overdose of cinnabar in drugs such as Bapuslsan, which is used as a sedative & for management of external intoxication in the Chinese population [21].

It must be aware of its toxic effects due to high Mercury content. Previous studies have shown that the insoluble form of HgS (or) cinnabar (10 g / 1water at about 20) can still be absorbed from GIT and liver [22].

Uses:

1. Cinnabar is used to be used commonly because of its physical properties in the art work of ancient times due to its interesting red colour.
2. It is used in the making of instruments traditionally it was used to recover gold sediments or streams and in used as a fungicide.
3. It is the principle ore of Mercury. So the Mercury is removed from this rock and used in the instruments such as thermometers and such.
4. It was also used as a powder called vermilion which, in small amounts could be used as food colouring.

Medicinal Uses:

1. Cinnabar is first rubbed with lemon juice for 3 hours. It is extremely efficacious drug in liver disorder.
2. Such as commencing Cirrhosis of liver, dyspepsia, chronic dysentery& similar other allied diseases, such as chronic diarrhoea.
3. Where the stools are deficient in bile.
4. In secondary syphilitic eruptions a powder composed of 2 parts of cinnabar and one of realgar is used for fumigation.
5. An ointment of cinnabar is applied to bring about the resolution of buboes.
6. In metal physical ore, cinnabar has positive effects on the immune system &blood.

Toxic symptoms of Cinnabar:

Most of the soluble salts of Mercury are absorbed slowly from the intestinal mucous membrane of the alimentary tract and produce their toxic effects.

After absorption the mercurial salts are excreted into the caecum and colon as sulphides in this form, Mercury is found in the fecal matter.

The long term use of cinnabar containing traditional medicines could result in renal dysfunction due to accumulation of Mercury in kidney.

Blurred vision due to accumulation of Mercury in brain is possible.

Skin allergic reaction may occur when cinnabar is used in tattoo dyes.

5. SIDDHA ASPECT OF *POORAM* (*Rasa Karpooram)*:

It is prepared by the combination of *Rasam* and salt [18a].

Method of preparation:

Ingredients

- Mercury – 336 gm
- Sulphur – 67.2
- Sodium Chloride – 650 gm

Procedure:

Sulphur is melted in a mud pot and Mercury is added to it and kindled well and there forms a black coloured pot. Brick stone powder is placed up to half of the level of a pot. Sodium Chloride is placed over it. Mercury Sulphur mixture is placed over the salt and sealed with mud pasted cloth. It is burnt for 12 hours with *kadakkini*. After it is cooled, the Mercurous chloride is found deposited on the upper pot and the same are collected.

Potency - Hot

Taste - Salt

Properties - Laxative, tonic, antiseptic and diuretic

General properties – It cures various types of throbbing pains. Hepatomegaly, pyrexia, Jaundice, Bacillary dysentery, dropsy, chronic ulcers, venereal diseases, indigestion, vomiting, diarrhoea, worm infestation, rheumatism, itching, constipation, scabies etc.

‘,ilthj #iy vh#iy Feke
njhilthio thjkhQ Nrhz-ailahNjh
nthfF urfwGunkhdNw asnthL ey
,fF ntyyjNjO ehs”

- பதார்த்த குண சிந்தாமணி

Medicinal uses:

- Poora kattu – Delirium associated with fever and delirium associated with constipation.
- Rasa Karpoora Kuligai – Scabies, Syphilis, Cervical Cancer etc

MODERN ASPECT OF HYDRARGYRUM SUBCHLORIDE

Other names

Calomel, Mercury (I) Chloride

Mercury (I) chloride is odourless solid and dense white or yellowish-white in colour. It is the principal example of a Mercury (I) compound. It is composed of Mercury and Chlorine (Mercury 84.98 % Chlorine 15.02 %). It is also referred to as the mineral horn quicksilver or horn Mercury [23].

Properties:

Molecular formula	-	Hg2Cl2
Molar mass	-	472.09 g/mol
Molecular Weight	-	472.09 gm
Appearance	-	White solid
Specific	-	7.27g/cc.
Density	-	7.150 g/cm3
Melting point	-	525 °C (triple point)
Boiling point	-	383 °C (sublimes)
Solubility in water	-	0.2 mg/100 ml
Hardness	-	1.5-2 - Talc-Gypsum
Refractive index	-	1.973
Fermion Index	-	0.25
Boson Index	-	0.75
Radioactivity	-	non radioactive
Other anions	-	Mercury (I) fluoride, Mercury (I) bromide
Other cations	-	Mercury (II) chloride

Medicinal properties:

Calomel was used internally to treat yellow fever during its outbreak in Philadelphia in 1793 and also used in the treatment of syphilis, until the early 20th century. It used as a laxative and disinfectant. During 18th century American Doctors used calomel to make patients regurgitate and release their body from "impurities". It was a common ingredient in teething powders, soaps and skin lightening creams in Britain up until 1954.

6. SIDDHA ASPECT OF *VEERAM (Savveeram):*

Other name

Perchloride of Mercury, Mercuric chloride

Perchloride of Mercury was first used as a therapeutic agent for venereal diseases during the middle of the eighteenth century in western countries. But for many centuries the Perchloride of Mercury has been used in India for the treatment of various disorders.

PROPERTIES:

Potency – Hot

Taste – Bitter

Actions – It has got body improving tonic and antiseptic.

General properties:

‘F d nkhL F l l q nfhba t d ÞyÞj jÞu l L

J d khq fÞr ngf f Q # i yNeha- t d i kAW

fhkÞag G z z hjÞa Neha f z l hwr t

t un d D d Q rhkÞehk j i j A r rhÞ”

It cures gastric ulcer, leprosy, throbbing pain and venereal diseases [18b].

Medicinal preparations:

- Mahaveera mezhgu-1-2 pulse grain dose for vatha diseases and venereal diseases.

- Veera mathirai-pills of pepper size for fever due to three humours
- Savveera Chendooram-for fever, delirium, vatha diseases, cholera etc.
- Veera rasa parpam-488mg dose
- Veera kalimbu-external application for ulcer
- Veera kulampu (Amirtha vennai)-applied on ulcers like cancer or carbuncle of chest, all swellings, boils etc.

MODERN ASPECT OF MERCURIC CHLORIDE:

Properties

Melting point	-	277ºc
Boiling point	-	302ºc
Density	-	5.44
Vapour pressure	-	1.3mm Hg
Refractive index	-	1.859
Storage temperature	-	Store at room temperature
Solubility	-	H_2O
Form	-	Powder
Water solubility	-	7.4g/100ml
Stability	-	Stable, but moisture sensitive and light sensitive decomposes in sunlight [24].

Preparation:

Mercuric chloride is obtained by the action of Chlorine on Mercury by addition of Hydrochloric acid to a hot, concentrated solution of Mercury (I) compounds such as the nitrate.

$HgNO_3+2HCl\ HgCl_2+H_2O+NO_2$

Heating a mixture of solid Mercury (II)Sulphate and Sodium Chloride also affords volatile $HgCl_2$, which sublimes and condenses in the form of small rhombic crystals.

Applications:

- Mercuric Chloride is a catalyst for the conversion of Acetylene to Vinyl Chloride, the precursor to Polyvinylchloride.
- It is used as a Depolarizer in batteries.
- It acts as a reagent in organic synthesis and analytical chemistry.
- It is being used in plant tissue culture for surface sterilisation of explants such as leaf or stem nodes.

PLANT INGREDIENTS:

GUNAPADAM ASPECT

7. *KATTRAZHAI (Aloe vera*)

Vernacular names

Eng: Indian aloes

San: kumara

Tel: kalabande

Mal: kattuvazha

Kan: kathalai

Hindi: ghikauvar

Other names: *Kanni, kumara.*

Parts used: Latex, Sap Juice, Root.

Taste - Slightly Bitter

Character - Coolant

Division - Sweet

Action

Tonic, Alterative, Purgative, Emmenogogue,

General properties:

‘nghyyh Nkfqfgk GOr#iy Fllufk

myyhH kjjkgfe juqFdkk vyyhktpl

NlFkhpfF nkhprrw fphprruK

khF khpf FkUzL”

-NjiuaHFzthflk

It cures Worm Infestation, Piles, Fistula, Delirium, and Gastritis [25].

Medicinal uses:

- The juice is used to reduce body heat and given as an adjuvant to Parpam and Chendooram.
- The juice is applied externally for inflammation and oedema.
- The juice mixed with gingely oil and applied over the head to induce sleep [26].

8.*Karummathai* (*Daturadiscolor*)

Vernacular names

Beng: kata dhatura

Hindi: kata dhatura

Duk: kata dhatura

Arab: jouzma leaved

Tel: nallaummetta

Other names: ummathai

Part used: leaf, flower, seed.

Taste: bitter

Character: heat

Division: pungent

Action: emetic

Anti spasmodic

Anodyne

Narcotic

General character: [27]

‘tpejpujq flLnkopd Nkdpjuq FllnkhL

tej tpaHg ghpgGkhwWq fhz-Kejg

ngUkjjQ nraRuj ijg NghfFq fagghq

fUkjjk ey%ypfhz”

Medicinal use:

- *Daturadiscolor* Leaf juice- 410gm
- Coconut oil- 816gm

This two are mixed well and boiled to get a dark consistency. Atthe end of boiling add omam 34 gm to get like resin form further add camphor 34 gm to cure all types of pain.

BOTANICAL ASPECT:

Kingdom - Plantae

Order - Solanales

Family - Solanaceae

Genus- Datura

Species - discolor

Health benefits of leaves

- The leaves of datura are good to relieve headache.
- The Vapour of datura leaves infusion is used to relieve arthritis such as rheumatism and gout.
- The burning leaf smoke of datura is good to treat asthma and bronchitis.
- The ethanol extract of datura is used as repellent against larva and mosquito.
- It is used to treat heart problems like palpitations and hypertension.
- Datura leaves juice is used to treat earache.
- Boils can also be overcome by applying datura leaves as poultice.

- Datura leaves are enriched with hyoscyamine and atropine can be used as mind altering drug [28].

3.2 LITERATURE REVIEW OF DISEASE

3.2.1. SIDDHA ASPECT OF DISEASE

Siddha system of medicine deals cancer and its treatment widely. In ancient Siddha literature cancer is explained as in the name of Putru which gives the direct meaning and as Arpudham and Vanmeegam. For the Purpose of diagnose and treatment following reference books evaluates great ideas about cancer.

1. Yugi Vaidhya Chintamani

2.AnubogaVaidhyaNavaneetham

3. Pulipani 500 [29]

4.Agathiya Vaidhya Vallathi [30]

The unique saint Pulipani dealt with different types of cancer in his Pulipani 500.

‘XNk d‰ FoÍgGwW Nah d‰gGwW
x s‰th d ,bgGwW f d dgGwW”

In this medical system of life, the cancerous growth and tumours are headed as Arputhaviranangal and Arputhakatttikal.

According to Yugimamunivarvaidhyasinthamani 800 I part, some kinds of cancer clarified under different systemic diseases. Yugiclassification ofdisease is compared with Western system of medicine by means of symptoms for quick and easy approach.

For example, Ukkarasoolai is understand as prostatic cancer

Vilperuvayiru is known as Testicular cancer

Mamisamagotharam and Kalperuvayiruas cancerous growth within abdomen.

To handle cancer effectively it is considered as Vippuruthi.

Types of *Vippuruthi*:

Vippuruthi is classified into seven types,

1. *Karppa Vippuruthi*
2. *Kuvalai Vippuruthi*
3. *Vatha Vippuruthi*
4. *Pitha Vippuruthi*
5. *Seththuma Vippuruthi*
6. *Santhu Vippuruthi*
7. *Oodu Vippuruthi*

Appearance

Causes of various classes look like one or more following appearance.

- Kazhalaikatti
- Spreading ulcer
- Initially like warts then grows and develops as turtle shell with oozing
- Hyper pigmentation of skin, affects hair follicles and destroys entire body.

Classification

Cancer classified into 3 types under its spreading nature (metastasis).

- Skin and its structures
- Muscles
- Blood vessels and bones.

Causes

- Vitamins and minerals deficiency
- Frequent sexual activities
- Prolonged starvation
- Excessive use of tobacco

- Rich intake of hot and spices
- Taking excessive amount of salt and pungent
- Taking large quantity of fish and meat
- Making sleep in day time.

Symptoms

Symptoms are varying depending on the particular type of cancer.

Yoniputru

As per Siddha literature Putru which affects the yoni (birth passage) also known as cervix of the uterus considered as karuppaikazhunthuputru.

Symptoms:

- Small grains like growth in cervix
- Honey like discharges
- Hardening of surface
- Profuse bleeding
- Constipation
- In some patients discharges with intolerable foul smell
- Oliguria and anuria. Administration of diuretics causes haematuria.

Discharges classified into 3 types

- Viscous yellowish discharge
- Yellow discharge with mucous
- Bloody discharge due to cervical non healing ulcer and cancer of cervix.

Yugivaidhyasinthamani classified the symptoms as follows.

Kuruthi yoni (Bleeding vagina)

‘jpwkhd Tgjjput kjpfq fhZk
njspahj ujjKld roeHg gharry
fwkhd EiuAlNd NehAz lhf
fbdkhaQ rijAlNd Fjjy fhZk
epwkhd kQrSld frNuhfe jhd
epiyahJ tyFypNy GONth nkjj
kykhd nrhyyJT KSjjhw Nghy
kQiQ ahdpwk Nghy krfFk ghNu” [31a].

Ulcers present in the vaginal wall, discharge with pus from the vagina and pelvic pain are the symptoms.

Mamisamagotharam

‘Nghffhd khkpre jhd tsHeJ kwp
nghUkpNa mbtapwwpy fyiyg Nghy
jhffhd rlejhD KyHeJ twwpj
jtpfFNk abffb jhdfz zHNjb
thffhd kJunkhop Fswpg Ngrp
thaTjh dbffbf FNkNy NehfFk
effhd kyry kpjpykhkprq fhZk
Neuhd khkprkNfhjuj jpNdNu” *[31b]*.

Lower abdominal pain, Foul smelling discharge, Presenting with blood.

Kuruthiseezh yoni

‘ghNujhd Ntj id Akpf T z lhFk

ghqfhd rOlNd ujjq fh Z k

rNujhd xOffK lNdehww khFk

rpjwpNa gyNgj t z z q fhlLk

NeNujhdp jkgjjpd] jhde j d dpy

nebjhd Nuhfj i jNktr nraAk

NtNujhd nrhd d gbrpfpr rhrhuk

tphpjjpl lhH A+fpK dp tpsffejhNd” [32].

Bleeding with mucous sometimes it is in multicolour, bad odour discharge, spread to whole uterus.

3.2.2. MODERN ASPECT OF THE DISEASE

CANCER

DEFINITION:

Cancer is a class of disease characterized by out of control cell growth which tend to proliferate and in some cases to metastasize (spread), known medically as malignant neoplasm with a broad group by 100 different types.

SYMPTOMS: [33]

- Persistent cough
- Change in bowel habits
- Blood in the stool
- Unexplained anaemia
- Breast lump or breast discharge
- Lumps in the testicles
- Change in urination
- Haematuria (blood in urine)
- Hoarseness
- Indigestion

- Unusual vaginal bleeding
- Unexpected weight loss
- Continued itching in anal or genital area
- Non healing sores
- Back pain, Pelvic pain.

.

VIRUSES IN HUMAN CANCER

Certain human malignancies are associated with viruses. Examples include Burkitt's lymphoma (Epstein-Barrvirus), Hepato cellular carcinoma (hepatitis virus), cervical cancer [Human Papilloma Virus (HPV)], and T cell leukaemia (retroviruses). The mechanisms of action of these viruses are varied but always involve activation of growth-promoting pathways or inhibition of tumour suppressor products in the infected cells. For example, HPV proteins E6 and E7 bind and inactivate cellular tumour suppressors' p53 and pRB, respectively. Viruses arenot sufficient for cancer development but constitute one alteration in the multistep process of cancer [34].

TUMOUR MARKERS [34a]

Table-2

TUMOUR MARKERS	CANCER	NON-NEOPLASTIC CONDITIONS
Hormones		
• Human chorionic Gonadotropin	Gestational trophoblastic disease, gonadal germ cell tumour.	Pregnancy
• Calcitonin	Medullary cancer of the thyroid.	
• Catechol amines	Pheochromocytoma.	
Oncofetal antigens	Hepato cellular carcinoma, gonodal germ cell tumour.	Cirrhosis, hepatitis
• Alpha fetoprotein		
• Carcino embryonic antigen	Adenocarcinoma of the colon, pancreas, lung, breast, ovary.	Pancreatitis, hepatitis, inflammatory bowel disease, smoking

Enzymes • Prostatic acid phosphates	Prostatic cancer	Prostatitis, prostatic hypertrophy
• Neuron specific enolase	Small cell cancer of the lung, neuroblastoma.	
• Lactate dehydrogenase	Lymphoma, Edwing's sarcoma.	Hepatitis, hemolytic anemia

TREATMENT:

- Surgery
- Radiation therapy
- Chemotherapy
- Immunotherapy
- Targeted therapy
- Hormone therapy
- Stem cell transplant, Precision medicine.

DISADVANTAGES OF TREATMENT:

Fatigue, hair loss, hearing loss, decreased sexual activity, diarrhoea, skin and nail changes, infections, anxiety, depression, fear, nausea, vomiting, lymph oedema.

CERVICAL CANCER

Definition: [35]

Cervical cancer is a type of cancer that occurs in the cells of the cervix. The cervix is the organ connecting the uterus and vagina. It is usually a slow-growing cancer that may not have symptoms but can be found with regular pap tests. This is a procedure in which cells are scraped from the cervix and looked at under a microscope. Cervical cancer is almost always caused by a human Papillomavirus infection.

Predisposing Factors:

- Average age 35-45 years.
- Coitus before the age of 18 years.

- Multiple sexual partners.
- Delivery of the first baby before the age of 20 years.
- Multiparity with poor birth spacing between pregnancies.
- Poor personal hygiene.
- Poor socioeconomic status.
- At one time, exposure to smegma from uncircumcised partners was considered an important factor, accounting for lower incidence of cancer cervix amongst the Jews and Muslims. Now it is realized that the incidence of Human Papilloma virus is low in circumcised men, and that is the reason of low incidence of cancer in their wives.
- Smoking and drug abuse including alcohol are immunosuppressive.
- Women with STD, HIV infection, herpes simplex virus 2 infection, Human Papilloma virus infection (16, 18, 31, 33) or condylomata have a high predisposition to cancer.
- Immuno suppressed individuals (following transplant surgery).
- Women with Pre invasive lesions.
- Women who do not come for regular health check-up and pap test [36].

PATHOLOGY:

Pap smear in invasive cancer shows tadpole cells and haemorrhage, necrosis in the background. It is customary to identify two types of cancers of the cervix. The first and more common variety is the epidermoid carcinoma. It arises from the stratified squamous epithelium of the cervix, and accounts for almost 80 percent of all cancers in the cervix. The second variety endocervical carcinoma arises from the mucous membrane of the endocervical canal, accounts for 20 percent of all cervical cancers. Histologically, 95 percent of cervical cancers are squamous carcinomas and only 5 percent are adenocarcinoma. This is because the columnar epithelium of the endocervix often undergoes squamous metaplasia.

Endocervical cancers of the cervix have recently increased in incidence because of prolonged use of oral combined contraceptive pills and progesterone's which have profound effect on glandular epithelium.

The malignant cells are endometroid, adenocarcinoma, clear cells and adenosquamous.

Squamous cell cancers of the ectocervix appear as proliferative growths, ulcers or as flat indurated areas. The common proliferative or cauliflower-like growth is vascular, friable and bleeds on touch. It undergoes ulceration and necrosis, which is associated with an offensive foul smelling vaginal discharge. The leucorrhoeal discharge is often blood stained. Histologically, the tumour is graded as well-differentiated or ill-differentiated. The endocervical growth remains confined to the cervical canal for a long time causing a barrel-shaped enlargement of the cervix, and only at a late stage it protrudes beyond the external cervical os and becomes visible.

The mode of spread of the cancer is by continuity or by continuity, by lymphatic spread or through vascular embolism to distant sites like lungs, liver, bones, kidneys and brain. Ovarian metastasis occurs in only 1 percent.

Clinical features:

Irregular mensus,

Menometrorrhagia,

Continuous bleeding,

Postcoital bleeding,

Leucorrhoea,

Blood stained or offensive discharge,

Cervix reveals a growth or an ulcer that bleeds on touch,

Bulkyuterus.

Differential Diagnosis:

Tubercular ulcer

Syphilitic ulcer

Fibroid polyp

Sarcomaof thecervix.

Staging of Cervical cancer

Stage I Carcinoma strictly conflicted to the cervix

IA Micro invasive carcinoma, not exceeding5.0mm.

IA1 Measured stromal invasion of less than 3.0mm in depth.

IA2 Measured stromal invasion between 3 and 5mm in depth.

IB Clinically visible lesion confined to the uterus

IB1 Clinically visible lesion 4.0cm.

IB2 Clinically visible lesion more than 4.0cm in dimension.

Stage II Cancer spread beyond the cervix, but not to pelvic wall or lower third of the vagina.

IIA Tumour without parametrical invasion.

IIB Tumour with parametrical invasion.

Stage III Tumour extends to the lateral pelvic wall, involves the lower third of vagina, and/or causes hydronephrosis or non functioning kidney.

Stage IV Tumour spread to the pelvic organs or distal metastasis.

IVA Tumour involves bladder and rectum.

IVB Widespread tumour with distant metastasis. [36a]

DIAGNOSIS

Biopsy

Investigations

- CT and MRI scans
- Positron Emission Tomography (PET)
- FDG-PET using F-18 fluoro-2-deoxy-D-glucose.

TREATMENT

STAGE IA1 –Conization therapy

STAGE IA2-Extended Hysterectomy and lymph node sampling.

STAGE IB and STAGE IIA

- Wertheim’s hysterectomy
- Schauta vaginal hysterectomy
- Primary radiotherapy
- Combined surgery and radiotherapy.

STAGE IIB, III AND IV

Chemo radiotherapy.

PALLIATIVE TREATMENT IN TERMINAL STAGE

- Pain relief with Morpia and Tramadol.
- Vomiting: correct dehydration and electrolyte balance.
- Diuretics and Spirolactone for as cites.
- Vaginal discharge reliefs with Bethadine douche.

3.2. PHARMACEUTICAL REVIEW:

Chendooram:

Definition:

Chendooram is a category of medicines made from metals or minerals (arsenicals or mercurial's or salts) by grinding them with specified juices or distillates or extractives and subjecting them to a process of sublimation or calcinations or burning or frying or exposing to insolation till the characteristic reddening of the product takes place. The *Chendooram* are said to retain their potency for 75 years

Method of preparation:

Usually two method of preparation are adopted in their processing though there are some exceptions and variants.

1. Sublimation by the sand – bath process

2. Calcination.

1. Sublimation by the sand - bath process (*KuppiErippu)*:

If the *Chendooram* has sulphur and Mercury as its components, sulfur is ground to a fine powder in the mortar and grinding should be continued with the addition of the given quantity of Mercury, till a black impalpable mobile powder is obtained. Only after this, the other ingredients are to be added.

In the conventional set up of the sand –bath sublimation contrivance, a heat resistant glass flask with a long neck is used as the container for the drug ingredients. Ceramic ware had also been in use. Before being put to use, these container are wound around with clay smeared cloth ribbons so as to give seven superimposed layers, leaving open the mouth of the flask. The flask thus encased should be kept for perfect drying of the covering.

It has been found in recent times that one could make use of the enameled iron bowls instead of glass flasks.

When using enameled iron bowls, two identical bowls of appropriate dimensions and capacity should be selected and checked for neat contact of rims when juxtaposed. Then small holes should be punched along the margins so that the two bowls could be fastened with a bonding wire (metallic). Then a perforation is made in the centre of the bottom of one of the bowls. Having prepared the bowls thus, they should be secured and bound by pasting the binding wire through the marginal holes. This would produce a capsule with a top orifice. Clay smeared cloth tape is wound around as would be done for the glass flask, leaving the central opening uncovered. This opening is the one through which the reaction going on inside is inspected by inserting a probe.

The sand – bath is set up by taking a wide earthen trough and spreading fine gravel or coarse sand at the bottom to a depth of two centimetres.

The capsule into which the drug ingredients are put is placed on the gravel or sand and is properly cantered. Then the sides packed with sands, leaving the top two centimetres unpacked and exposing the capsule. When using glass flasks, the neck should be just out of the sand. This setup is placed on the oven and heat is applied, by burning fire wood.

In the application of heat, there gradations are recognized. These three stages, mild, moderate and intense are best understood and mastered with some experience.

It is said that, if the flames are convergent and resemble a single tongue of flame as in a lamp, it is mild fire (*Deepakkini*). If several such tongues of flame lick the vessel and diverge like the flower of lotus, it is moderate (*Kamalakkini*). If the multiple

tongues of flame fill the oven and enrich the sand bath. It is the intense stage of fire (*Katakkini*).

These stages of fire should be manipulated and followed as prescribed in the method of preparation. In general, the heating is spread over three continuous days. In such cases, mild, moderate and intense stages are maintained for 24 hours each, in that order of succession.

According to the composition and amount of sulphur in the preparation, the mixture of drugs placed in the capsule will start melting sooner or later. Sulphur starts escaping first in the form of yellow vapour through the opening. Later it will start burning sending out a jet of blue flame. Just when the blue flame goes out if a long probe of steel wire is inserted into the orifice and drawn out the portion that enters the container will show a whitish coating. If the sulphur is still present and not totally burnt out, the probe will have a black sticky coat, when there is no blackening of the probe and when whitish coat indicating should be closed and heating continued for one or two hours and then the heat withdrawn and the setup is allowed to cool by itself.

When the setup has cooled down, the capsule containing the medicine is taken out and the clay tape winding cut out. The material that has sublimed in upper bowl is gently tapped with suitable beater or lifted with a spatula. The sublimate collected should be finely ground in a mortar.

If the glass flasks have been used, the flask is carefully broken, open to collect the medicine that has sublimed in around the neck.

2. Calcination (*Putam)*:

The powder is ground in a *Kalvam* with specified fluids for a specified time. The paste is made into small discs and dried. They are put in earthen saucers (*man agal*) covered with another and the edge well sealed with mud cloth. It is allowed to dry. The cups are placed in the middle of cow – dung cakes and burnt. For *Putams,* generally pits of various depths and circumferences are made in the ground. Half of the pit is covered with cow – dung cakes. The earthen cups are placed and it is covered again with cow-dung cakes. The fire is put in the middle of the heap on all the four sides so that there would be uniform heat from all the sides.

All the metals and other ingredients are taken after the usual purification. In specified cases, specific purification (*Suddhi*) is mentioned; otherwise, it is to be taken as general method of purification for the drug as mentioned in Materia- Medica books.

Other method of preparations:

1. Prepared without heating (*Araippu Chendooram)*

2. Prepared by open heating (*Erippu or Varuppu Chendooram)*

3. Prepared by applying heat in the range close to 100◦c (*LaguPuda Chendooram).*

Specifications for *Chendooram*

1. *Chendooram* is red in nature, well fine in particle size and tasteless.
2. With suitable adjuvant they possess therapeutic values.
3. They are said to retain their potency for 75 years [36].

ANALYTICAL SPECIFICATIONS OF *Chendooram* [37]

Table-3

S.no	Test
1.	Description-colour, odour
2.	Identification-chemical
3.	Particle size-200 to 300
4.	Loss on drying at 105ºc
5.	Total ash
6.	Acid-insoluble ash
7.	Water soluble ash
8.	Assay of element (s)
9.	Siddha specifications
10.	Lustreless
11.	Fine enough to enter the cervices of finger
12.	Floats on water
13.	Smokeless
14.	Tasteless
15.	Irreversible

3.3. PHARMACOLOGICALREVIEW

Our great Siddhars explained many medicinal preparations to cure the life threatening cancer disease.

Herbal Origin:

Compound herbal preparations

Pills

- Asuvagandhathi vadagam

Chooranam

- Garudakodi Chooranam
- Karanthai Chooranam
- Kukilathy Chooranam
- Megaroga Chooranam
- Vallathy Chooranam

Mineral and Metal Origin:

Siddhars identified and worked on many metal and mineral preparations which had anti cancer activity.

Preparations

Pills

- Mahakodasuzhi mathirai

Parpam

- Kariya parpam [38]
- Naga parpam [39]
- Rasa parpam
- Kandhaga poora parpam [40]
- Sootha parpam [41]
- Thalaga parpam
- Sanda rasa parpam

Chendooram

- Gowri chinthamani Chendooram [42]
- Gandhaga Chendooram [40a]
- Linga Chendooram [43a]
- Kala mega narayana Chendooram [44]
- Navachara Chendooram [45a]
- Karuvanga Chendooram [45b]
- Narayana Chendooram [46]
- Pavalavanga Chendooram [41a]
- Sandamarutha Chendooram [42c]
- Asthabairava Chendooram
- Naga Chendooram [47]
- Rasa Chendooram
- Thambira Chendooram [44a]

Pathangam

- Guru pathangam [48a]
- Veera rasa pathangam
- Putrupathangam [59]

Thylam

- Singi thylam [51a]
- Pupudhakkar mega thylam
- Pachai thylam [51b]
- Sengathari thylam [52a]
- Vippuruthiennai [52c]
- Mega rasangaennai [52b]
- Chinthamaniennai [59a]
- Sengottai thylam [50a]
- Visharajanga thylam [50c]
- Meganathiennai
- Magasanthanathy thylam [54]
- Puda thylam

Nei

- Kukkilnei [54]
- Thengainei
- Vallarainei

mezhugu

- Korosanai mezhugu
- Gandhaga mezhugu [40b]
- Kanagalinga mezhugu [48b]
- Guru sanjeevi mezhugu [48c]
- Valai rasa mezhugu [49a]
- Veera mezhugu [50a]

Others

- Madhusmeegi rasayanam
- Kulirntha pachai
- Veelai seelai [51a]
- Rana pugai [51b]
- Chitravallathi lehiyam

Siddha drugs for Yoniputru:

Pills

- Chitramoola kuligai [47b]

Chooranam

- Karanthai chooranam [50b]

Parpam

- Thambira parpam [55]
- Rasa parpam [56]
- Velli parpam [57]
- Gandhaga parpam
- Karuvanga parpam

Chendooram

- Panchapadana Chendooram [58]
- Muthu Chendooram [41b]
- Swarnapushpa rasa Chendooram
- Namachivaya Chendooram

Thylam

- Chitramoola Nei [60]
- Vallarainei [50c]
- Perungaya ennai
- Magathu ennai
- Gandhaga thylam
- Meganathi thylam
- Sathrusangara ennai

Mezhugu

- Amirthanandhi mezhugu
- Gandhaga mezhugu [40c]
- Korosanai mezhugu
- Rasagandhi mezhugu [61]
- Vithu rasa mezhugu [62]

Kattu

- Poora kattu [43b]

Pathangam

- Linga pathangam [48d]

Kirutham

- Vallarai kirutham [49b]

Others

- Gandha rasa villai [47a]
- Soolai kudori [47b]
- Megampokum Rasagandhi [63]

CLASSIFICATION OF ANTI -CANCER DRUGS [64] Table-4

1. Alkylating agents	
Nitrogen mustards	Mechlorethamine, cyclophosphamide, ifosfamide, chlorambucil, melphalan, bendamustine
Ethylenimines	Thio-Tepa, Altretamine
Alkyl sulfonate	Busulfan
Nitrosoureas	Carmustine, streptozocin
Triazine	Dacarbazine, temozolomide
Methyl hydrazine	Procarbazine
2. Antimetabolites	
Folate antagonist	Methotrexate (amethopterin), pemetrexed
Purine analogues	6-Mercaptopurine, thioguanine, pentostatin,
Pyrimidine analogues	fludarabin, cladribine.
	5-Fluorouracil, floxuridine, capecitabine, cytarabine (cytosine arabinoside) gemcitabine
3. Natural and semisynthetic products	
Antibiotics	Actinomycin-D (Dactinomycin), daunorubicin, doxorubicin, bleomycin, mitomycin-C, mithramycin
Epipodophyllotoxins	Etoposide, teniposide
Camptothecins	Topotecan, irinotecan
Taxanes	Paclitaxel, docetaxel
Vinca alkaloids	Vincristine, vinblastine, vinorelbine
4. Miscellaneous	Hydroxyurea, cisplatin, I-asparaginase, imatinib, bortezomib, thalidomide, monoclonal antibodies.
5. Hormones and their antagonists	Glucocorticoids, androgens, antiandrogens, oestrogens, antioestrogens, progestins, aromatase, inhibitors.
6. Biological response modifiers	Interferon alpha, interleukin 2, amifostine, haematopoietic growth factors

ANTI-CANCER DRUGS

DRUGS	MOA	USES
Cyclophosphamide	Forms reactive derivates, alkylates DNA and important groupscytotoxicity	NHL CLL, breast, ovarian cancer, soft tissues sarcoma, Wilms tumour, Rhabdomyosarcoma.
Busulfan	Same as above	CML
Methotrexate	Folate antagonist-MTX, polyglumates decreases DHFR-inhibits protein synthesis.	Choriocarcinoma, NHL, breast, bladder, head, and neck cancer, osteogenic sarcoma.
Mercaptopurine	Purine analog-incorporated into DNA and RNA- breaks into DNA, inhibits DNA synthesis.	AML
5-Fluorouracil	Pyramidine analog-incorporated into DNA and RNA- inhibits DNA synthesis, inhibits TS.	Colorectal, anal, hepatocellular, gastric, ovaries, head, and neck cancers.
Actinomycin-D	Inhibits DNA dependent RNA synthesis.	Wilmstumour, Ewings tumour rhabdomyo sarcoma, chorio carcinoma, kaposis and soft tissue sarcoma, immuno suppressant.
Bleomyocin	Bind iron, generates free radicals-breaks into DNA	Testicular tumours, Head and neck cancer, HL and NHL

Daunorubicin & Doxorubicin	Bind DNA and inhibits topoisomerase.	Testicular tumours. Head and neck cancer.
Etoposide	Inhibits topoisomerase II	Lung and Gastric cancer.
Topotecan	Inhibits topoisomerase I	Lung and Ovarian cancer.
Vinblastine	Inhibits mitosis	Breast cancer, Kaposi's sarcoma
Vincristine	Inhibits mitosis	Neuroblastoma
Cisplatin	Active form inhibits DNA synthesis.	Lung, breast, bladder, testis, ovarian, head and neck cancers.

Common adverse effects of anti cancer drugs:

- Bone marrow depression
- GIT-stomatitis, glossitis, esophagitis.
- Alopecia (loss of hair)
- Reduced spermatogenesis in men and amenorrhoea in women
- Nausea and vomiting are immediate side effects
- Hyperuricaemia (increased plasma uric acid levels) leads to renal failure
- Teratogenicity
- Carcinogenicity (cause secondary cancer).

SCREENING METHODS:

Cancer is one of the thrust area for which effective drugs at comfortable prices are not available as yet probably due to lack in understanding the cancer Patho physiology. For such a dreadful disease anti cancer drugs have been developed from a variety of sources ranging from natural products (plants and microbes) to synthetic molecules. One of the cause of treatment failure is the development of resistance to anticancer agents [65].

The widely used drugs which are called as cancer chemotherapeutic agents have many side effects such as bone marrow suppression, alopecia, nausea and vomiting.

This necessitates screening of a large number of compounds .For this purpose both in-vitro and in-vivo models are employed for systematic screening of an anticancer drugs [66].

IN-VITRO METHODS

In-vitro testing is a potential chemotherapeutic agent .

Advantages:

1. More cost effective

2. These are easier to manage

3. These are less time consuming

4. Small quantities and large number of compounds can be tested.

Disadvantages:

1. Pharmacokinetics in determining drug effects cannot be evaluated.

2. Growing Solid tumour is poor compared to in-vivo method.

ASSAY [66]

For energy metabolism and autophagy

FAD assay

ATP assay

Lysosome detection

Mitochondrial membrane potential assay

Reactive oxygen species test

For nuclear signalling, DNA damage and cell proliferation

P^{53}assay

Topoisomerase II assay

P^{21}assay

Cell proliferation assay

Mdm2 assay

Clonogenic assay

For inflammation, angiogenesis and metastasis

Cytokine and chemokine assay

STAT 1, 2, 3, 6 assays

COX-2 activity assay

Cell migration assays

LDL uptake assay

For apoptosis, pyroptosis and necrosis

Caspase 1 assay

Bax assay

Cytolysis assay

Calpanin assay

For cancer signalling pathway and phenotype

ERK assay

cAMP assay

IN VIVO MODELS

Many animal species develop cancers spontaneously and are valuable for understanding the biology of sporadic cancer development in humans. The major use of spontaneous cancer models is to compare the biology with human; these animals are increasingly valuable for cross-comparison of response or resistance to the same clinical agents used for patients. [66a]

Animal models

1. Mouse cancer models
    a. GEM-Genetically Engineered mouse Models
    b. Inbred mice (systematic sibling mating)
    c. Transplantation models

        Allograft models (syngeneic tumour tissues derived from same genetic mouse)

Xenograft models (actual human cancer cells or solid tumours are transplanted into host mouse)

d. Carcinogen induced and spontaneous models

1.Digestive system cancer induced by polycyclic aromatic

2.Chemically cancer induced by Cadmium and Arsenic

3.Radiation-skin cancer by ultraviolet radiation, leukemic changes by ionizing radiation.

2. Rat cancer models

a. Genetically altered rats

1. Treat embryos with DNA damage causing chemical mutagen, Frequently N-ethyl-N-nitrosurea (ENU) is used.

2. Insertion of mutagenesis strategies (Retro viruses)

3. Transgenic strategies (pronuclear injection of DNA)-quickly developed and more effective models.

b. Inbred rats.

3. Other laboratory animal models

a. Hamster

b. Rabbits

c. Zebrafish

4. Other animal models

a. Dogs

b. Cats

c. Goats

d. Horses

e. Pigs.

Cervical cancer cell lines

- HeLa (HPV 16)
- SiHa (HPV 18)
- C33A (HPV negative)
- CaSKi

Induction of cervical cancer in animal models:

1. Cervical neoplasia is induced in mouse by an extract of varicella zoster virus infected cells (HPV or Herpes simplex virus type 2 DNA)
2. Genomic HSV 2 DNA was isolated from isolated from infected Herpes cells and separated from host cell DNA by cesium Chloride density gradient centrifugation. The DNA was applied to mouse cervix for period of 80 to 100 weeks. Should examine monthly to detect abnormalities.

4. MATERIALS AND METHODS

SELECTION OF THE DRUG:

For this present study, the Herbo-mineral formulation *Namachivaya Chendooram* was taken as the compound drug preparation for cervical cancer mentioned in the classical Siddha literature *Prana Rakshamirtha Sindu Vaithya Ratna Sangaragam*, written by *T.R.Mahadeva Pandidhar* [68].

INGREDIENTS:

Main ingredients:

- *Rasam* (Mercury)
- *Saathilingam* (Cinnabar)
- *Veeram* (Perchloride of Mercury)
- *Pooram* (Calomel)
- *Thaalagam* (Yellow Arsenic)
- *Kaantham* (Magnetic oxide of Iron)

Associate Plant juices:

- *Kattralai (Aloe vera)*
- *Karuoomathai (Datura discolor)*

COLLECTION OF THE DRUG:

- The raw materials were purchased from Tampcol Raw drug store, Anna Hospital Campus, Arumbakkam, Chennai.
- Fresh plant materials were collected from in and around alagarkovil, Madurai.

IDENTIFICATION AND AUTHENTICATION OF DRUG:

The raw materials were identified and authenticated by Botanist and Gunapadam experts, Government Siddha Medical College, Arumbakkam, Chennai. A specimen sample of each raw material has been kept in the department for future reference.

PURIFICATION PROCESS

Purification process was done as per classical Siddha literature [18c].

1. Purification of Mercury

Materials Required:

- Mercury - 35 gm
- Brick powder - 100 gm
- Turmeric powder - 100 gm
- Acalypha juice (*Acalypha indica*) - 1.3 litre

Procedure:

Mercury was triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the Mercury was boiled with the juice of Indian Acalypha till the juice completely evaporates.

2. Purification of Mercurous Sulphide (Cinnabar)

Materials Required:

- Mercurous Sulphide - 35 gm
- Lime juice - 150 ml
- Cow's milk - 150 ml
- Indian *Acalypha* juice - 150 ml

Procedure:

Equal proportion of lemon juice, cow's milk, *Acalypha* juice were mixed with each other. Cinnabar was processed with above juice and cow's milk to get purified form.

3. Purification of Mercuric Chloride (Per Chloride of Mercury)

Materials required:

- Mercuric Chloride - 35 gm
- Camphor - 100 gm
- Tender coconut water - 200 ml

Procedure:

Camphor was mixed with tender coconut water and placed in a mud pot. Mercuric Chloride was tied in a cloth and tied in a cross bar and hanged without touching the water and the pot was burnt for half an hour to get purified Mercuric Chloride.

4. Purification of Hydrargyrum Sub Chloride (Calomel)

Materials required:

- Hydrargyrum Sub Chloride - 35 gm
- Betel leaf *(Piper betel*) - 8.75 gm
- Pepper(*Piper nigrum*) - 8.75 gm
- Water - 1.3 litre

Procedure:

The Poultice made of betel leaf and pepper was taken and dissolved in water. Calomel was tied in a cloth and immersed in the liquid hung from the cross bar and heated. After the water was reduced to ¾ of its volume, the calomel was taken out washed with water and dried to get purified form.

5. Purification of Arsenic trisulphide

Materials required:

- Cow's urine - 1.3litre
- Indian *Acalypha* juice - 325 ml
- Limestone - 325 gm

Procedure:

Arsenic trisulphide is bundled and kept immersed in the above mixture and heated to get purified.

6. Purification of Magnetic Oxide of Iron

Materials required:

- Magnetic oxide of Iron - 35 gm
- Lemon juice - 150 ml
- Rice Vinegar - 150 ml
- Butter milk - 150 ml

Procedure:

The Magnetic oxide of Iron was soaked in lemon juice, fermented sour rice water and sour butter milk for three days each. It was then dried in sun shine and washed. Thus purified form was obtained.

Aloe vera:

The sap of *Aloe vera* was washed with running water seven times. Then the sap was mixed with *kadukkai* powder (*Terminalia chebula*) to obtain the juice.

Datura discolor*:*

Clean the leaves of *Datura* and juice is made.

INGREDIENTS (Fig.1.1 to 1.8)

Fig no: 1.1 YELLOW ORPIMENT

Fig no: 1.2 MERCURY

Fig no:1.3 MAGNETIC OXIDE OF IRON

Fig no: 1.4 CINNABAR

Fig no: 1.5 MERCURIC CHLORIDE

Fig no: 1.6 CALOMEL

Fig no: 1.7 Datura discolor

Fig no:1.8 *Aloe vera*

PREPARATION OF THE DRUG (Fig.2.1 to 2.3)

Fig no: 2.1 PROCESS OF CHENDOORAM

Fig no: 2.2 AFTER IGNITION BY DEEPAKINI

Fig no: 2.3 FINAL PRODUCT OF CHENDOORAM

4.1. Preparation of *Namachivaya Chendooram:* [67]

- Purified Mercury - 35 gm
- Purified Cinnabar - 35 gm
- Purified Perchloride of Mercury - 35 gm
- Purified Calomel - 35 gm
- Purified Yellow Arsenic - 35 gm
- Purified Magnetic oxide of Iron - 8.75 gm

Plant materials:

- *Aloe vera* juice - 450 ml
- *Datura discolor* juice - 250 ml

Procedure:

In the very first step, all the purified raw materials except Mercury were powdered separately and mixed with Mercury thoroughly. The mixture was placed in a*kalvam* (stone mortar) then juice of *Karuoomathai (Datura)*was added and grounded well for 12hours(4saamam).

Then the grounded materials were made into pellets which were kept in sunlight until dry and placed in a mud pot and covered with another mud pot. The two pots were kept close to each other and sealed with seven layered mud smeared cloth. This was subjected to ignition process (*deepakini*) for 12 hours.

After the process gets completed the pellets were kept in a stone mortar again triturated with *Kattrazhai (Aloe)*juice for 12hours(4*saamam*).The same ignition process followed for 12 hours. This process was repeated twice.The pellets were separated, dried from the mud pot gets subjected to cool down. The fine dark colour powder on sublimation (*Chendooram)* was obtained in the top of the pot removed by a clean spoon. Finally the product was ground well in the stone mortar. Then the powdered material was labelled as *Namachivaya Chendooram* (*NMC*).

Storage: The drug was preserved in a clean air tight glass container.

Dosage: *Oruarisiedai* (weight of one rice grain) (65mg)

Form of Medicine: Chendooram

Route: Enteral

Time of Administration: Twice a day

Adjuvant: Honey

Shelf life of the drug:75 years.

Indications:

Venereal diseases, **cervical cancer**, penile cancer.

Analysis as per AYUSH guidelines

1. Floating on Water:

A pinch of *Chendooram* gently placed on the still surface of water in a vessel, did not sink immediately. It was found that the *Namachivaya Chendooram* particles floated over the surface of water indicated lightness of the trial drug.

2. Lines on fingers:

Chendooram in well prepared form should be as fine powder. When taken between thumb and index finger, the fine powder will fill up the lines of the finger print. A pinch of *Namachivaya Chendooram* was taken in between the thumb and index finger and rubbed. It was found that the *Namachivaya Chendooram* entered into the lines of the finger and was not easily washed out from the lines, confirmed its fineness.

3. Irreversible reaction:

The well prepared *Chendooram* does not get reversible to its metallic state when heated with a mixture of cane jaggery, hemp powder, ghee and honey. A pinch of *Namachivay*a *Chendooram* was taken and mixed with cane jaggery, ghee and honey. It was observed that *Namachivaya Chendooram* did not reverse to its metallic state.

4. Tasteless:

The well prepared *Chendooram* should be completely tasteless. Presenceof any taste like sweet or bitter indicate incomplete preparation which needed another Calcination process. When a small amount of *Namachivaya Chendooram* was kept on the tip of the tongue, no specific taste was found.

5. Lustreless:

If any shining particle is present in *Chendooram*, it indicates that the *Chendooram* is not manufactured properly and contains unchanged substances like minerals, metals and other toxic substances. There should be no shining particles present in the well manufactured *Chendooram*. The *Namachivaya Chendooram* was taken in a petri bowl and observed for any lustre in daylight through magnifying glass. No lustre was observed in the *Chendooram*.

DRUG STANDARDISATION:

Standardisation of drug means confirmation of its identity, determination of its quality, purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological evaluations [69].

STANDARDIZATION OF THE DRUG *NMC:*

Standardization of drugs helps to prove its identity and determination of its quality and potency. Standardization of the Herbo-mineral formulation is based on the qualitative and quantitative analysis through Physico-chemical investigations and instrumental analysis. The Physico-chemical analysis of the prepared Herbo-mineral drug have been done at Central Research Institute, Arumbakkam, Chennai and elemental analysis have been done at IIT, Chennai. (FTIR, SEM, ICP-OES, XRD)[68]

4.2.1. PHYSICO CHEMICAL ANALYSIS:

Physico chemical studies of the trial drug have been done according to the WHO guidelines.

pH value:

Potentiometrically pH value is determined by a glass electrode and a suitable pH meter.

Loss on Drying:

Loss of weight expressed as percentage resulting from water by volatile matter of any kind that can be driven off under specified conditions .The test is carried out on a well mixed sample of the substance, if the substance is in the form of large crystals, reduce the size by rapid crushing to a powder form.

The powdered drug is dried in the oven at 100- 105°C to constant weight.

Action on heat:

A small amount of the sample is taken in a dry test tube and heated gently. If strong white fumes evolve indicate the presence of Carbonate.

Flame test:

A small amount of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame. Appearance of bluish green flame indicates the presence of Copper.

Ash Test:

A filter paper is soaked into a mixture of sample and Cobalt Nitrate solution and introduced into the Bunsen flame and ignited. Appearance of yellow colour flame indicates the presence of Sodium.

DETERMINATION OF TOTAL ASH

The residue remaining after incineration is the ash content of the drug.

TOTAL ASH VALUE:

Total ash method is used to measure the total amount of material remaining after incineration.

ACID SOLUBLE ASH:

It is the residue obtained after boiling the total ash with dilute HCl and igniting the remaining insoluble matter.

WATER SOLUBLE ASH:

It is the difference in weight between total ash and residue after treatment of total ash with water.

BIO-CHEMICAL ANALYSIS

The bio-chemical analysis was done to identify the acid and basic radicals present in the *NMC*.

Preparation of extract

5g of NMC was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

4.2.2. PRELIMINARY BASIC AND ACIDIC RADICALS

Test for basic radicals

1. Test for Potassium

To a pinch of the *NMC* 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of *NMC* extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium:

To 2ml of *NMC* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of *NMC* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the *NMC*, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. **Test for Iron (Ferrous)**

The *NMC* extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the *NMC* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the *NMC* extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of *NMC* extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate**.**

10. Test for Copper

a. A pinch of *NMC* was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of *NMC* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

To 2m1 of the *NMC* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic

To 2 ml of the *NMC* extract 2ml of sodium hydroxide solution was added and brown or red precipitate formation was noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the *NMC* extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *NMC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. **Test for Phosphate**

The *NMC* extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. **Test for Carbonate**

The *NMC* extract was treated with conc.HCl and observed forth appearance of effervescence.

5. Test for Fluoride & Oxalate:

To 2ml of *NMC* extract 2ml of dilute acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate:

To 1 gm of the *NMC*, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

4.2.3. ANTI-MICROBIAL ACTIVITY

AVAILABILITY OF MICROBIAL LOAD:

Enumeration of bacteria by plate count – agar plating technique

The plate count technique was one of the most routinely used procedures because of the enumeration of viable cells by this method.

Principle:

This method is based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies therefore is the same as the number of organisms contained in the sample.

Dilution:

A small measured volume is mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilution is usually made in multiples of ten. A single dilution was calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the diluent}}$$

Requirements:

- Sample or Bacterial suspension
- 9 ml dilution blanks (7)
- Sterile petri dishes (12)
- Sterile 1 ml pipettes(7)
- Nutrient agar medium (200 ml)
- Colony counter.

Procedure:

1. Label the dilution blanks as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}.
2. Prepare the initial dilution by adding 1 ml of the sample into a 9 ml dilution blank labeled 10^{-1} thus diluting the original sample 10 times.

3. Mix the contents by rolling the tube back and forth between hands to obtain uniform distribution of organisms.
4. From the first dilution transfer 1 ml of the suspension while in motion, to the dilution blank 10^{-2} with a sterile and fresh 1 ml pipette diluting the original specimen to 100 times.
5. From the 10^{-2} suspension, transfer 1 ml of suspension to 10^{-3} dilution blank with a fresh sterile pipette, thus diluting the original sample to 1000 times.
6. Repeat this procedure till the original sample has been diluted 10,000,000 times using every time a fresh sterile pipette.
7. From the appropriate dilutions transfer 1ml of suspension while in motion, with the respective pipettes, to sterile petri dishes. Three petri dishes are to used for each dilution.
8. Add approximately 15 ml of the nutrient medium, melted and cooled to 45^0c, to each petri dish containing the diluted sample. Mix the contents of each dish by rotating gently to distribute the cells throughout the medium.
9. Allow the plates to solidity.
10. Incubate these plates in an inverted position for 24-48 hours at 37^0c.

Observation:

Observe all the plates for the appearance of bacterial colonies. Count the number of colonies in the plates.

Calculate the number of bacteria per ml of the original suspension as follows:

$$\text{Organisms per millimetre} = \frac{\text{Number of colonies (average of 3 replates)}}{\text{Amount of plated} \times \text{dilution}}$$

4. 2. 4. SOPHISTICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

DEFINITION:

FTIR offers quantitative and qualitative analysis for organic and inorganic samples. Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan

samples for many different components. FTIR is an effective analytical instrument for detecting functional groups [69].

APPLICATIONS:

- Quantitative Scans, Qualitative Scans
- Solids, Liquids, Gases
- Organic Samples, Inorganic Samples
- Unknown Identification
- Impurities Screening
- Formulation
- Pharmaceuticals.

INSTRUMENT DETAILS

Fig no: 3.1 FTIR INSTRUMENT

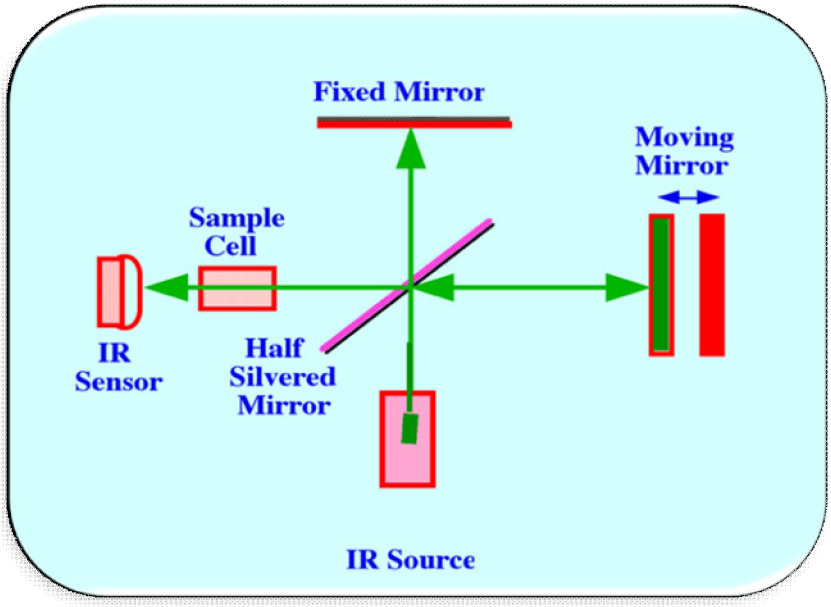


Fig no: 3.2 FTIR MECHANISM

Model	**: Spectrum one: FT-IR Spectrometer**
Scan Range	**: MIR 450-4000 cm-1**
Resolution	**: 1.0 cm-1**
Sample required	**: 50 mg, solid or liquid.**

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It is an excellent tool for quantitative analysis.

In FT-IR infrared is passed from a source through a sample. This infrared is absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there is no two unique molecular structures producing the same infrared spectrum. It is recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

FT-IR is the most advanced and the major advantage is its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated [70].

SEM (SCANNING ELECTRON MICROSCOPE)

DEFINITION

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects [71].

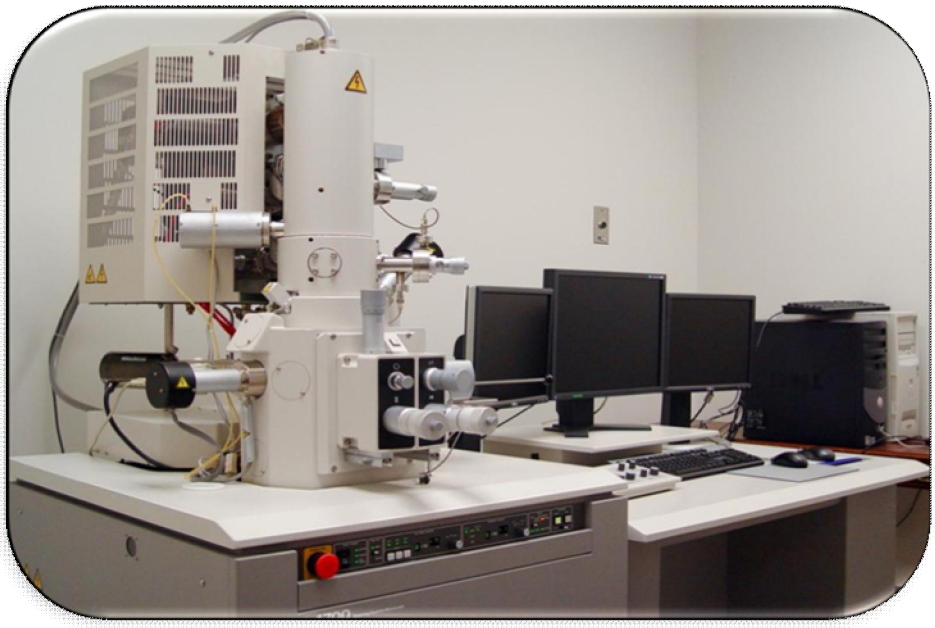


Fig no: 3.3 SEM INSTRUMENT

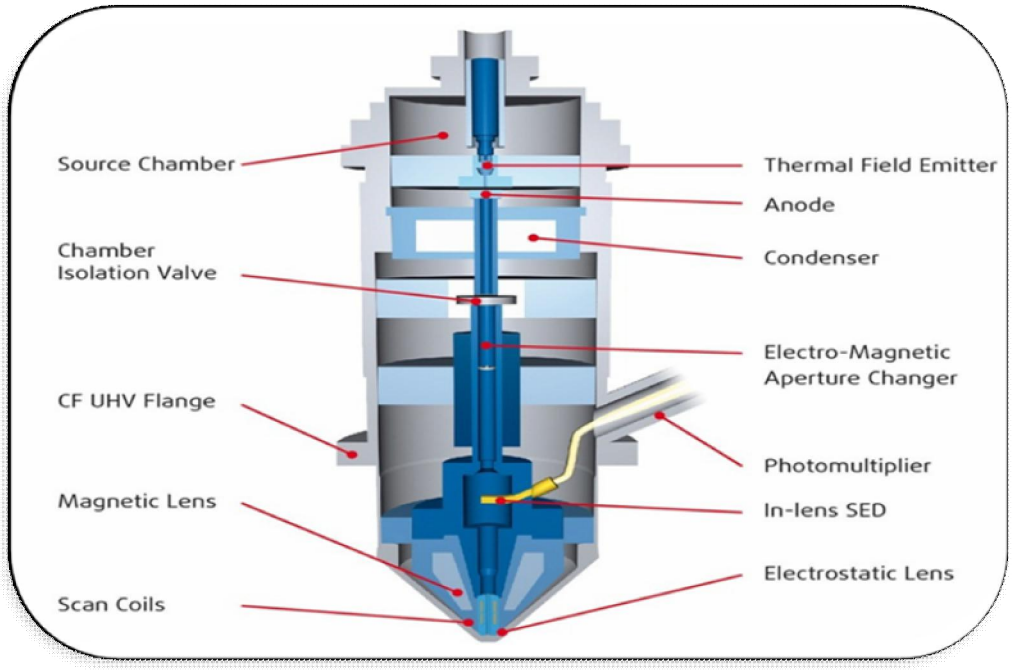


Fig no: 3.4 SEM MECHANISM

SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

- External morphology (texture)
- Chemical composition (when used with EDS)
- Orientation of materials making up the sample

The EDS component of the system is applied in conjunction with SEM analysis to:

- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results
- Identify foreign substances that are not organic in nature and coatings on metal
- SEM Analysis with EDS – qualitative and semi-quantitative results
- Magnification – from 5x to 300,000x
- Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height
- Materials analysed – solid inorganic materials including metals and minerals.

THE SEM ANALYSIS PROCESS

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high-energy electron beam is focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it is collected by an appropriate detector [72].

The types of signal produced by a scanning electron microscope include

- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

XRD (X-ray powder diffraction)

DEFINITION

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of

unknown solids is important to studies in geology, environmental science, material science and biology.

Fig no: 3.5 XRD instrument

APPLICATIONS:

- Characterization of crystalline materials [73]
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to:

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
    - determining lattice mismatch between film and substrate and to inferring stress and strain
    - determining dislocation density and quality of the film by rocking curve measurements
    - measuring super lattices in multilayered epitaxial structures
    - determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Limitations

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation. Powder less than ~10 μm(or 200-mesh) in size is preferred
- Place into a sample holder or onto the sample surface.

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig no:3.6 ICPOES-INSTRUMENT

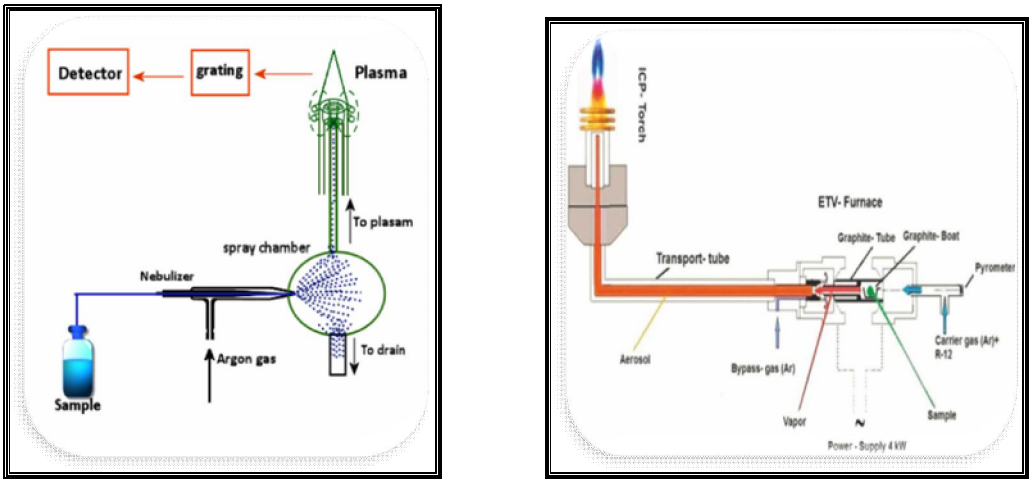


Fig no:3.7 ICPOES-MECHANISM

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP)

Principle:

An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone (8,000– 10,000ºC). The analysts are heated (excited) in different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample.

The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant (such as the concentration of ferrous iron or Ferric Iron), only total essential concentration is analysed by ICP-OES [74].

Application:

The analysis of major and minor elements in solution samples.

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- Probes the outer electronic structure of atoms.

Mechanism:

In plasma emission spectroscopy (OES), a sample solution is presented into the core of Inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardizationvalues.

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis.

100 mg NMC was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes until the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

The digested sample solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.

4.2.5. TOXICOLOGICAL STUDIES

ACUTE ORAL TOXICITY – OECD GUIDELINES – 423 [75]

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423.

IAEC No: IAEC/XLIV/30/CLBMCP/2014,Baid Metha College of Pharmacy, Duraipakkam, Chennai.

INTRODUCTION:

The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. Morbid animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

Animal: Healthy Wistar albino female rat weighing 200–220 gm

Studies carried out at three female rats under fasting condition, signs of toxicity were observed for every one hour for first 24 hours and every day for about 14 days from the beginning of the study.

PRINCIPLE:

It is the principle of the test that is based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing is needed − dosing of

three additional animals with the same dose − dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

METHODOLOGY

Selection of animal species:

The preferred rodent species is rat, although other rodent species may be used. Healthy young adult animalsof commonly used laboratory strain Swiss albino rat was obtained from Animal house of King's institute, Guindy, Chennai. Females should be nulliparous and non-pregnant. Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within±20 % of the mean weight of the animals. The studies were conducted in the animal house of C.L. Baid Metha College of pharmacy, Duraipakkam Chennai.

Housing and feeding conditions:

The temperature in the experimental animal room should be 22ºC (+3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hrs light, 12 hrs dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be grouped and tagged by dose, but the number of animals per cage must not interfere with clear observations of each animal.[77]

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

EXPERIMENT PROCEDURE:

Administration of doses

Namachivaya Chendooram prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It was given in a single oral dose by gavage using a feeding needle.

Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed.

Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 16–18 h prior to the administration of the test suspension. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Number of animals and dose levels

Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 6 animals (3 animals per step).

Duration of Study	:	48 hrs
Evaluation	:	14 Days

Limit test

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hrs.
- Special attention: First 1-4 hrs after administration of drug, and

- It is observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Body weights will be recorded at day 1, day 2, 7 and 14 of the study

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necropsy findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *Namachivaya Chendooram with* 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline is pharmacologically inert and easy to employ for new drug development and evaluation technique.
(Schlede E., Mischke U., Diener W. and Kayser D 1992;66: 455-470)

REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *NAMACHIVAYA CHENDOORAM* ON RATS – (OECD-407 guidelines) [76]

Justification for Dose Selection

The results of acute toxicity studies in Wistar albino rats indicated that *Namachivaya Chendooram* was non-toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected for the study were100mg/kg, 200 mg/kg and 400 mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.[78]

Preparation and administration of dose

Namachivaya Chendooram at three doses respectively was suspended in 2 ml of 2% CMC in distilled water. It was administered to animals at the dose levels of 100, 200 and 400 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

Ten rats (Five Male and Five Female) were in each group randomly divided into four groups for dosing up to 28 days. Animals were allowed acclimatization period of 7

days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight*:* Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Clinical signs: All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality*:* All animals were observed twice daily for mortality during entire course of study.

Functional Observations: At the end of the 4^{th} week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations: Following laboratory investigations were carried out on day 29 in animal fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Blood chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 rpm. for 10 minutes. On 28th day of the experiment, 24 h urine samples were collected by placing the animals in the metabolic cage with free access to tap water but no feed was given.

The urine was free from faecal contamination. Toluene is used as a preservative while collecting the sample. The sediments present in the urine were removed by centrifugation and the collected urine was used for biochemical estimations. On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to

obtain the serum. Serum was stored at 20 °C until analysed for biochemical parameters.

Haematological Investigations: Blood samples of control and experimental rats was analyzed for haemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations: Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of Glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Urine analysis: Urine samples were collected on end of treatment for estimation of normal parameters. The estimations were performed using appropriate methodology.

Necropsy*:* All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, brain, heart, and lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of animal on sacrifice day (g)}} \times 100$$

Histopathology: Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 400 mg/kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-Eosin. The organs included heart, kidneys, liver, ovary, pancreas, brain, spleen and stomach, of the animals were preserved they were subjected to histopathological examination.

Statistical analysis: Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by Dunnet's multi comparison test using a computer software programme GRAPH PAD INSTAT-3 version.

4.2.6. PHARMACOLOGICAL ACTIVITY

IN-VITRO ANTICANCER ACTIVITY DETERMINATION BY MTT ASSAY

HeLa (cervical cancer cells) was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbeccos modified Eagles medium (Gibco, Invitrogen).

The HeLa cell line was cultured in 25 cm2 tissue culture flask with DMEM supplemented with 10% FBS L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT (4,5-dimethylthiazol-2-yl) assay method.

Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of plant extracts and compound stock:

1 mg of sample/compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Cytotoxicity Evaluation:

After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl

were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator.

Cytotoxicity Assay by Direct Microscopic observation:

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization.

After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 200µl of MTT Solubilization Solution DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilise the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of control group}} \times 100$$

ANTI-TUMOUR ACTIVITY

Cell culture

The human cervical carcinoma a cell line, SiHa, used in the study was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were grown in DMEM containing 2m ML-glutamine supplemented with 10% fetal bovine serum and100U/ml of penicillin-streptomycin. The cellswere incubated in a humidified 5 %CO_2 incubator at37°C.

Cell growth analysis

SiHa cells were seeded at a density of 1 × 105 cells/ml in24-well plates in triplicates. Next day, the cells were dosed with different concentrations of *NMC* (0, 10, 20, 40 and80 μg/ml) and grown for 24, 48 and 72 h. The cells were harvested and counted for viability using trypan blue dye exclusion method.

Colony formation assay

The cells were plated at a seeding density of 1 × 103 cells/ ml in 6-well plates. After 24 h, the cells were exposed to various concentrations of *NMC*: 0, 10, 20, 40, and 80 μg/ ml. Plates were incubated at 37°C in a5%CO_2 incubator for one week. This was followed by fixing the colonies with 4%paraformaldehyde and staining with0.5%crystal violet [77].The colonies were photographed with SonyDSC-S75cyber-shotcamera.

Soft agar assay

Control SiHacells (5×10^3cells/ml) as well as cells treated with different concentrations of *NMC* (10-80 μg/ml) were mixed at 40°C with 0.35%agarose(DNA grade, GIBCOBRL,CA,USA) in culture medium and gelled at room temperature for 20min over a previously gelled layer of 0.5% agarose in culture medium in 6-wellplates. After incubation for 10days, colonies were photographed directly using an Axiovert200M microscope (CarlZeiss, Germany) and counted.

Measurement of Apoptosis

The cells were plated at a seeding density of 5 × 105 cells/ well and treated with different concentrations of *NMC* (0-80 μg/ml). After 24 h of treatment, the cells were harvested and washed with PBS twice. Cells were stained with Annexin V-FITC following the manufacturer's instructions (Annexin V-FITC apoptosis kit #3, Invitro- gen) and analyzed for apoptosis by FACS using CellQuest Software.

Statistical analysis

All experiments were performed in triplicates and repeated at least five times and the data were presented as mean ±SD. Statistical analysis was conducted with the Sigma Stat3.5program (Systat Software, Inc.) using one- way ANOVA. The level used for comparisons was α=0.05.

ANTI OXIDANT ACTIVITY

DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of *Namachivaya Chendooram* extracts was determined by using DPPH assay according to Chang e tal.(2001).The decrease in the absorption of the DPPH solution after the addition of an anti-oxidant was measured at 517nm.Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle

1,1Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an anti-oxidant (H- A) can be written as,

$$(DPPH)+ (H-A)\rightarrow DPPH-H +(A)$$

Anti-oxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the anti-oxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100mlof ethanol.

Working procedure

Different volumes (1.25-20µg/µl) of *Namachivaya Chendooram* extracts were made upto 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. There action mixture was incubated in dark condition at room temperature for 20min.After 20min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the *Namachivaya Chendooram* extracts was calculated using the follow

$$\% \text{ inhibition} = \frac{\text{Control-test}}{\text{Control}} \times 100$$

5. RESULTS AND DISCUSSION

One of the Siddha Herbo-mineral formulations, *Namachivaya Chendooram* had been exposed to several modern scientific studies to establish its efficacy to scientific people and public. Literary collection, Physicochemical and elemental analysis, toxicological studies and pharmacological studies are done to justify the anticancer activity of *NMC* against cervical cancer.

From review of literature

Discussion on Gunapadam review

- The poem for general properties of processed quicksilver directly indicates its anticancer nature.
- *Pooram* by its formulation as pills directly used as an anti cancer drug.
- General property of *Veeram* used to kill certain cancer growth.
- As per Siddha classical text, *Kaantham* by its herbo-mineral formulation indicates anti cancer property against cervical cancer.

Discussion on modern drug review

- Mercury helps to destroy the cancer cells and reduces the tumor growth [78].
- Mercuric Chloride has cyto toxicity effect [79].
- The bioactive compounds of *Aloe vera* which are potential to inhibit cancer cell proliferation [80].
- Arsenic exhibits anti -cancer activity [81].
- *Datura discolor* contains anti-oxidant activity.
- Juice of *Aloe vera* possesses anti-oxidant property [82].

Discussion on pharmaceutical review

Chendooram:

75 years of shelf life denotes its long time efficacy.

Being very fine particles it increases the therapeutic effect.

Discussion of pharmacological review

The cell lines for anticancer activity were HeLa and SiHa. They are the genomes of HPV 16 and HPV 18 respectively. These HPV 16 and HPV 18 are responsible for 93% of Cancer cervix [83].

So, the analysis of pharmacological activity through HeLa and SIHA cell lines are the novel methods for validation. They explained about the effective anticancer activity of *NMC*.

Discussion on materials and methods

The selection of trial drug was taken from the book ***Prana Raksha Mirtha Sindhu Vaithya Ratna Sangaragam,*** Written by **T.R. Mahadeva Pandidar**, was approved by the Department of AYUSH as Per Classical Siddha literature.

The ingredients were bought from the authenticated vender and they were identified and authenticated by the experts in Post Graduate Department Gunapadam, GSMC, Chennai. So the ingredients were perfect and original.

The preparation of medicine was done at the well-equipped lab of the Post Graduate Department Gunapadam. So the principles of GMP were adhered during process.

The analytical parameters were conducted at registered and licensed laboratories only. Thus the result of *Namachivaya Chendooram* under various analytical procedures show accuracy of it.

The Siddha Herbo-mineral formulation *Namachivaya Chendooram* had been subjected to various studies for its scientific validation and safety assessment. Literary collections, physicochemical and Elemental analysis, Toxicological study, Pharmacological studies are done to prove its efficacy.

Results of Siddha Standardization

Table-5

	Parameter	**Results of ideal *chenduram***	**Results of SC**	**Interpretation**
1.	Colour	Reddish	Reddish orange	Chendooramcolour.
2.	Floating on Water	Floats on water	Floats on water	Lightness of drug.
3.	Finger Print Test	Impinged in the furrow of fingers	Impinged in the furrow of fingers	Indicates fine particles of powder.
4.	Lustre	Lusterless	Lusterless	Change of specific metallic character of raw material after incineration
5.	Taste	No specific taste,	No specific taste	Change of specific metallic character of raw material after incineration

Colour:

It is brownish red in colour. The absence of shining indicates there is no free form of metals.

Floating on water:

Namachivaya Chendooram floats on water. It is due to its less specific gravity. So, it possesses the property of *chendooram.*

Finger print test:

Namachivaya Chendooram impinged on the crevices of finger. This indicates the particles are fine and it is in micro size.

Lusterless& tasteless:

It is lusterless and tasteless

Physical characterization of *Namachivaya Chendooram*

Table-6

S.no.	Parameter	Result
1.	Colour	Brownish red in colour
2.	State of the drug	Powder
3.	Consistency	Fine powder
4.	Solubility	Sparingly soluble in water, DMSO. Well soluble in acids (Hcl and H_2So_4)
5.	Sense on touch	Fine
6.	Sense on taste	Tasteless
7.	Sense of smell	No significant smell is observed

Results of Physical Parameters

Table-7

S.NO	Parameter	Result
1.	Specific gravity	0.976
2.	Ph	3.24
3.	Flame test	+ve
4.	Ash test	-
5.	Particle size	Completely passes through sieve no.120
6.	Loss on drying at 105 degree Celsius	5.767%
7.	Total ash	14.88%
8.	Water soluble ash	2.57%

Solubility

Solubility is the major factor that controls the bioavailability of a drug substance.

It is useful to determine the form of drug and processing of its dosage form.

The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability [84].

NMC is soluble in major solvents and sparingly soluble in some solvents Proves that its efficiency of solubility in the stomach indirectly, increasing the bio availability.

P^H value

Namachivaya Chendooram shows acidic P^H.

The P^H level plays a role in enzyme activity by maintaining the internal environment thus regulating the homeostasis.

It is also important factor for drug absorption [85]. Because of the acidic nature, the drug is more readily absorbed in an acidic medium like stomach which enhances the bioavailability of the drug.

Specific gravity

The trial drug *Namachivaya Chendooram* shows low specific gravity compared to water. This indicates its nature of absorption.

Flame test

Water blue color flame was found which indicates presence of amount of arsenic.

Loss on drying

Loss on drying (LOD) gives the total amount of volatile content and moisture (water) present in the drug.

The stability of a drug and its shelf-life are dependent on moisture content.

Moisture increase can adversely affect the active ingredient.

Low moisture content- drug could get maximum stability and better shelf life.

Since the drug has low loss on drying, the moisture content is less which is suitable for medicine preparation.

Ash values

Total Ash value

Low total Ash value indicates the trial drug contains plant organic derivatives. It is not subjected to calcinations process.

Acid insoluble ash

Lower the acid insoluble value better will be the drug quality [86]. The drug ensures a low value of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

Water soluble ash

Decreased water soluble ash value (2.57 %) indicates easy facilitation of diffusion and osmosis mechanisms.

Bio Chemical analysis

Results of acid and basic radical studies

Table-8

Parameter	Result
Test for Potassium	Positive
Test for Calcium	Positive
Test For Magnesium	Negative
Test For Ammonium	Negative
Test For Sodium	Negative
Test for Iron (Ferrous)	Negative
Test For Zinc	Negative
Test For Aluminium	Negative
Test For Lead	Negative
Test for Copper	Negative
Test For Mercury	Positive
Test for Arsenic	Positive
Test for Sulphate	Positive
Test for Chloride	Positive
Test for Phosphate	Negative
Test for Carbonate	Negative
Test for fluoride &oxalate	Negative
Test For Nitrate	Negative

DISCUSSION:

The Biochemical analysis for basic radicals of *NMC* shows the presence of Calcium, Mercury and Arsenic.

The Biochemical analysis for acidic radicals of *NMC* shows the presence of Sulphate and Chloride.

The Presence of these radicals helps *NMC* for its therapeutic effect.

AVAILABILITY OF MICROBIAL LOAD

Fig no: 4.1 BACTERIAL LOAD 10^{-6}

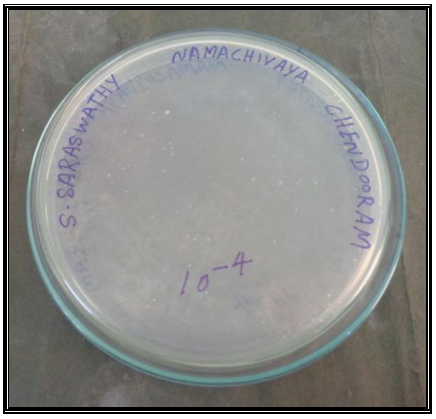


Fig no:4.2 10^{-4}

Fig no:4.3FUNGAL LOAD 10^{-3}

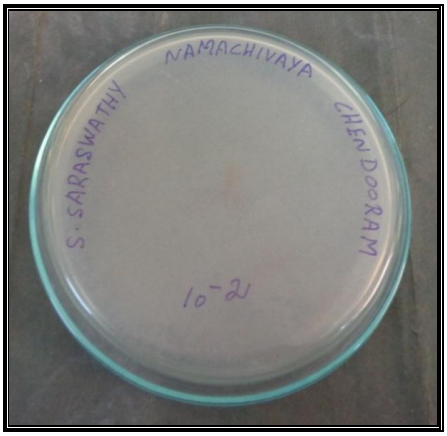


Fig no:4.4 10^{-2}

DISCUSSION:

- This is one of the herbo-mineral formulations which are prepared by using plant materials they are prone to contamination. The contamination of herbal

drugs by micro organism not only cause bio deterioration but also reduces the efficacy of drugs.

- The toxic effect produced by microbes makes the herbal drugs to give no response for human consumption because the contaminated drug may develop unwanted disease instead of disease being cured.
- Here, the contamination of *chendooram* has been examined by bacterial and fungal load.
    - Total bacterial load in 10^{-4} dilution is 22 and in 10^{-6} dilution is 13.
    - Total fungal load in 10^{-2} dilution is nil and in 10^{-3} dilution is nil.
- Here, the contamination of *NMC* is within the WHO norms. Hence, the drug is collected, prepared, stored and packed and decontaminated prior to formulation.

FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY)

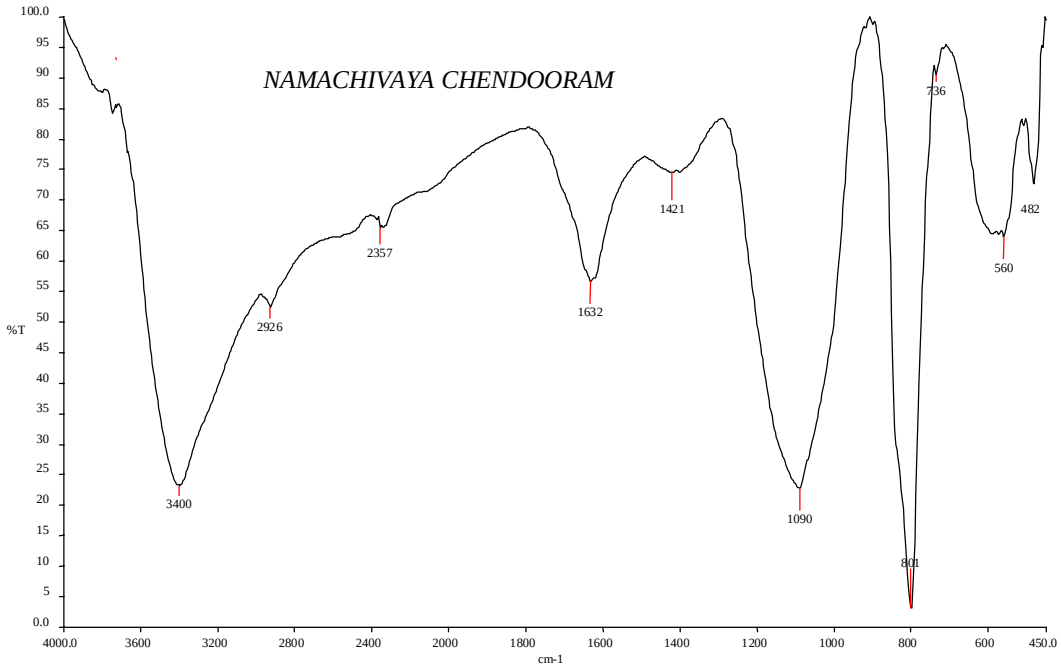


Fig no: 5 Peak values by FTIR

Interpretation Table-9

Absorption peak cm-1	Stretch	Functional Group
3400	O-H(stretch, H-Bonded), N-H stretch,	Alcohols and amide
2926	C-H stretch, O-H stretch	Alkane and Acid
2357	C=O stretch	Carboxyl group
1632	-C ≡C- stretch	Alkene
1421	-C-H bending, C=C stretch	Alkanes, Aromatic
1090	-C-N(stretch), C-F stretch, C-O stretch	Amine, Alkyl Halide, Ether
801	=C-H	Alkene
736	=C-H bending, C-Cl Stretch	Alkene, Alkyl Halide
560	C-Br Stretch	Alkyl Halide

The wave numbers from 4000cm-1 to 1500cm-1 gives details for identification of functional group.

The wave number from 1500cm -1 to 400 cm-1 provides particulars about molecular fingerprint.

The above result showed the presence of functional group like alcohols, Alkanes, amides in *Namachivaya Chendooram.*

They may be responsible for the presence of anticancer action of *NMC* in cervical cancer.

Amides

Amide derivatives of Benzene- sulfonanilide, a Pharmaceutical composition are used in cancer treatment [87].

The lead molecule of these compound was methane sulfonamide, a cyclooxgenase (COX) inhibitor. They act as efficient anti tumour agents.

OH

OH group of *NMC* has higher potential towards inhibitory activity against microorganisms.

Phenols

Phenols of *NMC* possess highly Anti-Oxidant property which enhances its effect against the disease

The effect of phenols is currently of great awareness due to their anti-oxidative and possible anti carcinogenic activities.

Free radicals react easily with phenols to abstract the hydrogen atom from the OH group. Phenolic acids and flavanoids also work as reducing agents, free radical scavengers and quenchers of single oxygen formation (Ali Ghasemzadehet al.2011)

Phenolic acids components take part important roles in the control of cancer and other human diseases.

Phenols are the most important groups of secondary metabolites and bioactive compounds. Hydroquinone is one of the phenolic group inhibits the free radical reactions. (cho7Alchohol HTI) They are also an antioxidant substance capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer.

Phenols and flavanoids possess diverse biological activities, for example, antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic and antidepressant activities.

Alkanes

Alkane derivative like bis (4-amino-5-mercapto-1, 2 ,4-triazol-3-yl) possess anti-cancer activity [88].

Carboxylic acid

Benzene-poly-carboxylic Acid Complex (BP-CI) is a novel anticancer complex against human cancer cells.

Docosahexaenoic acid (DHA) is an omega-3 fatty acid. Its structure is a carboxylic acid (-oic acid) with a 22- carbon chain (docosa-is Greek for 22) and six (hexa-) cis double bounds [89].

DHA was revealed to increase the efficacy of chemotherapy in prostate cancer cells and a chemo protective effect in a mouse model was reported.

It may also be used as a non- toxic adjuvant to increase the efficacy of chemotherapy.

In mice, DHA was found to reduce growth of human colon carcinoma cells
The cytotoxic effect of DHA was caused by decrease in cell growth regulators.

Ether:

Certain ether lipids such as 1-0-octadecyl-2-0 methyl-rec-glycero-3-phosphocholine represent a new class of anti -neoplastic agents. These ether lipids have been shown to be cytotoxic for a wide variety of tumors.

SEM (SCANNING ELECTRON MICROSCOPE)

The following image is done by 10000X magnification via 500µm aperture shows maximum depth focused.

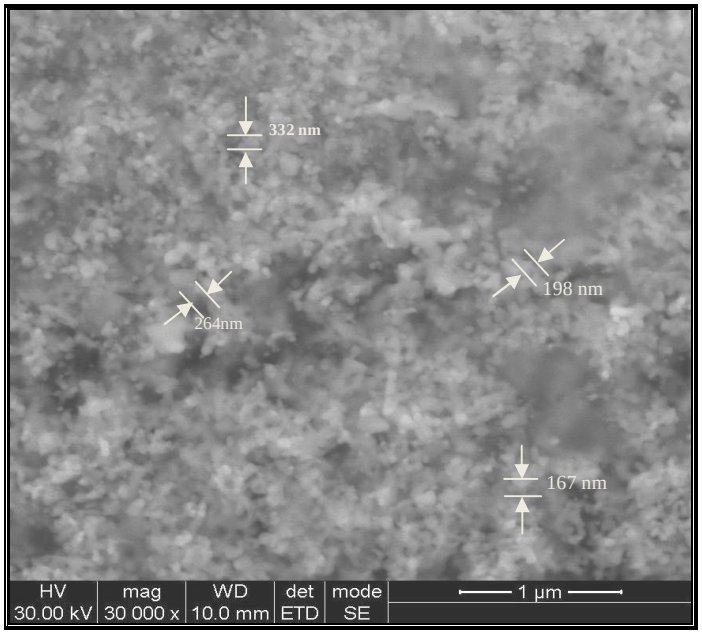


Fig no: 6 Showing nano particles in SEM image of 1µm

Discussion on SEM reports

- Nanoparticles, according to the American Society for Testing and Materials (ASTM) standard definition, are particles with lengths that range from 1 to 100 nm in two or three dimensions.

Advantages of nano particles:

- Enhancing solubility of hydrophobic drugs,
- Prolonging circulation time,
- Minimizing nonspecific uptake,
- Preventing undesirable side effects,
- Improving intracellular penetration,
- Specific cancer targeting [90].
- The test drug *Namachivaya Chendooram* contains Nano particles.
- Nano particles present in the drug results in a better bioavailability and facilitates absorption.
- Nanotechnology a promising way from cancer management towards cancer elimination.
- The particles of nano size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

ICP-OES RESULTS AND DISCUSSION

ICP-OES interpretation of *NMC*
Table-10

S. no	Elements	Detected levels
1.	Arsenic	03.214mg/L
2.	Phosphorus	08.541mg/L
3.	Iron	51.330mg/L
4.	Mercury	0.943mg/L
5.	Sulphur	122.514mg/L
6.	Lead	BDL
7.	Cadmium	BDL
8.	Sulfur	BDL

Discussion on ICP-OES

From the above results, the heavy metals Cadmium and Lead were found below detection level. Iron, Mercury and Arsenic are observed within the permissible limits.

Iron

Iron deprivation could be an excellent therapeutic approach: (i) dietary iron restriction markedly decreases tumour growth in rodents [91].

Sulphur

Sulphur is commonly used in Asia as an herbal medicine to treat inflammation and cancer. Organic sulfur has been studied on oral and other cancers and has been found to have remarkable benefit in anti-cancer therapy.

Hence the safety of the drug *Namachivaya Chendooram* is ensured.

XRD (XRAY DIFFRACTION)

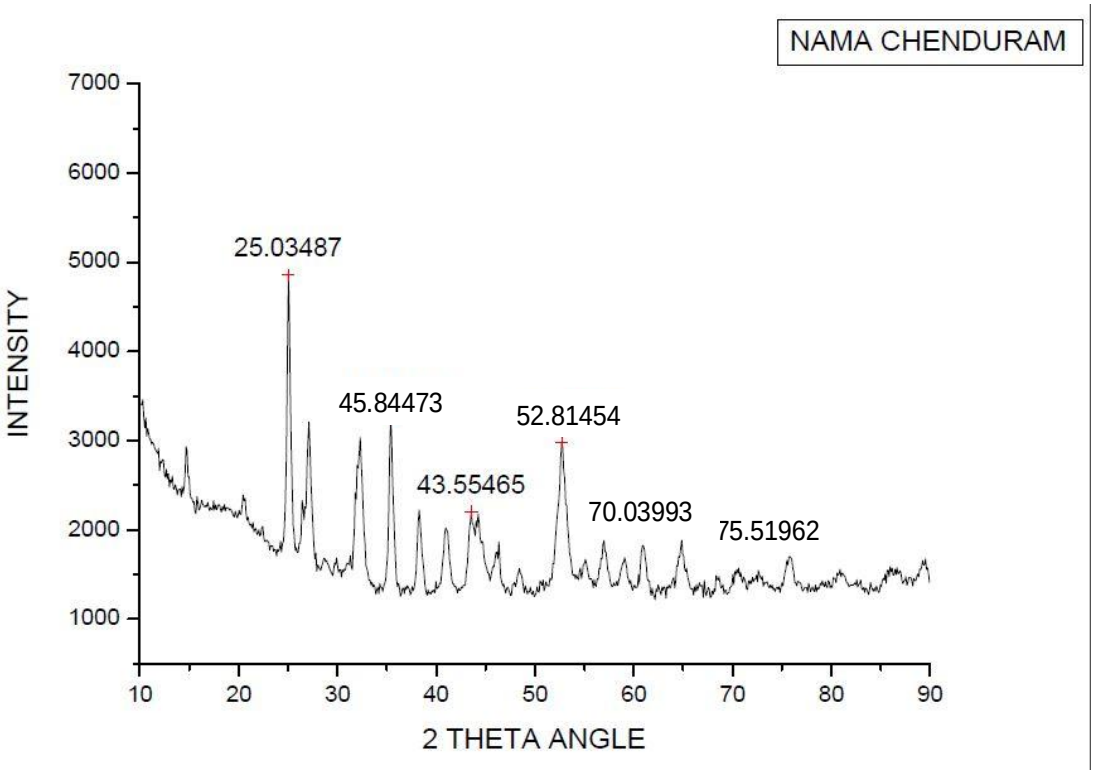


Fig no: 7 XRD Interpretation

Discussion

The crystalline structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The nano particles may enhance bio absorption of the drug.

XRD pattern of *Namachivaya Chendooram* shows the good crystallinity after calcinations process. The major diffraction peaks are identified after XRD analysis *NMC* concluded that HgS in nano crystalline range(31 -56nm) is association with organic molecules probably plays an important role in making it biocompatible and non toxic at therapeutic doses. Other elements present in *NMC* act as additional supplement and possibly helps in increase the efficacy of the formulation [92].

ACUTE ORAL TOXICITY

Dose finding experiment and its behavioral Signs of Toxicity for *Namachivaya Chendooram.*

Observation done:

Table-11

Group	Day
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant colour change
Piloerection	Normal
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle gripness	Normal
Rearing	Mild
Urination	Normal

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
200	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Table-12

Behavioural Signs of Toxicity for *NMC*

1. *Alertness 2.Aggressiveness 3. Pile erection 4. Grooming 5.Gripping 6. Touch Response 7.Decreased Motor Activity 8.Tremors 9 Convulsions 10. Muscle Spasm 11. Catatonia 12.Musclerelaxant 13.Hypnosis 14.Analgesia15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19 Respiration 20. Mortality*

Acute toxicity Discussion:

- In the acute toxicity study, the rats were treated with different concentration of *NAMACHIVAYA CHENDOORAM* from the range of 5mg/kg to 200mg/kg.
- This dose level did not produce signs of toxicity, behavioral changes, and mortality in the test groups as compared to the controls when observed during 14 days of the acute toxicity experimental period.
- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.
- In acute toxicity test the *NAMACHIVAYA CHENDOORAM* was found to be non toxic at the dose level of 200mg/ kg body weight

SUB-ACUTE ORAL TOXICITY 28 DAYS REPEATED DOSE STUDY IN RATS

Body weight (g) changes of rats exposed to *Namachivaya Chendooram*

Table-13

Dose (mg/kg/day)	*Days*				
	0	**7**	**14**	**21**	**28**
Control	120.59±0.92	122.79±0.87	123.52±1.18	127.24±1.12	131.25±1.05
20	123.48±1.37	128.29±1.43	127.9±1.50	129.8±1.24	130.98±1.73
40	127.57±1.07	132.51±1.25	133.62±1.84	134.7±2.43	137.7±1.43

Values are expressed as mean ± S.E.M; N=3; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs control.

Effect of *Namachivaya Chendooram* on Organ weight in rats

Table-14

Organ	Control	20 mg/kg	40 mg/kg
Liver (g)	3.07±0.20	3.37±0.13	2.83±0.16
Heart (g)	0.32±0.04	0.36±0.04	0.41±0.05
Lung (g)	0.28±0.05	0.33±0.04	0.42±0.03
Spleen (g)	0.25±0.06	0.37±0.03	0.43±0.06
Brain (g)	0.37±0.05	0.45±0.05	0.49±0.05
Kidney (g)	0.76±0.05	0.91±0.01	0.69±0.03

Values are expressed as mean ± S.E.M; N=3; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs control.

Effect of *Namachivaya Chendooram* on Hematological parameters in rats

Table-15

Parameter	Control	20 mg/kg	40 mg/kg
RBC ($\times 10^6/mm^3$)	8.29±0.43	8.99±0.55	10.32±0.27
PCV (%)	49.66±0.77	50.92±0.54	56.16±3.78
Hb (%)	15.13±0.39	16.16±0.13	14.46±0.32
WBC ($\times 10^3/mm^3$)	11.75±0.85	14.04±1.08	13.22±0.41
Neutrophils (%)	23.29±0.73	26.17±0.69	25.33±0.38
Eosinophills (%)	4.1±0.23	5.2±0.32	5.7±0.27
Lymphocyte (%)	85.5±0.46	90.3±0.49	86.83±0.23
Platelets ($\times 10^3/mm^3$)	425.73±1.35	493.62±14.8	467.52±26.73

Values are expressed as mean ± S.E.M; N=3; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs control.

Effect of *Namachivaya Chendooram* on Biochemical parameters in rats

Table-16

Parameters	Control	20 mg/kg	40 mg/kg
Glucose (mg/dl)	108.63±0.81	112.74±0.72	111.07±0.53
BUN (mg/dl)	22.06±1.55	25.60±0.57	27.46±0.69
Creatinine (mg/dl)	0.85±0.07	0.93±0.02	0.91±0.04
SGOT (U/L)	74.35±1.23	78.91±1.14	75.22±1.13
SGPT(U/L)	27.07±0.84	31.40±0.39	29.03±0.35
ALP (U/L)	104.63±1.14	109.07±0.95	106.21±0.45
Protein (g/dl)	8.58±0.68	10.67±0.49	12.39±0.46
Albumin (g/dl)	5.34±0.40	7.91±0.60	6.12±0.55
Total Cholesterol (mg/dl)	93.21±1.16	102.28±0.66	97.02±0.91
Triglycerides (mg/dl)	52.58±1.56	62.50±1.06	58.07±0.59

Values are expressed as mean ± S.E.M; N=10; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs control.

Effect of *Namachivaya Chendooram* on Urine parameters in rats

Table-17

Parameters	Control	20 mg/kg	40 mg/kg
Colour	Yellow	Yellow	Yellow
Transparency	Clear	Slightly turbid	Slightly cloudy
Specific gravity	1.01	1.02	1.04
PH	7.2	7.8	8.1
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
RBCs	Nil	Nil	Nil
Epithelialcells	Nil	1-2cells/HPF	Nil
Casts	Nil	Nil	Nil

Sub-Acute Toxicity Discussion:

- The dose selected for the sub acute toxicity study was 20mg, 40mg/kg of *NAMACHIVAYA CHENDOORAM*.
- All the animals were free of intoxicating signs throughout the dosing period of 28 days.
- No physical changes were observed throughout the dosing period.
- No mortality was observed during the whole experiment. No abnormal deviations were observed.

- No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits.

- The weights of organs recorded that shows mild differences in the treatment when compared to control group. This indicates that *Namachivaya Chendooram* induce mild changes in liver and kidney but not toxic to rest of the organs.

- There was slight changes were observed in hemoglobin (Hb), red blood cell (RBC). No significant changes in white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

HISTOPATHOLOGY EXAMINATION

- Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for hematological and blood chemical analyses were taken from common carotid artery.

- All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

PHARMACOLOGICAL STUDY

Anti-cancer activity CELL LINE: HeLa

Table-18

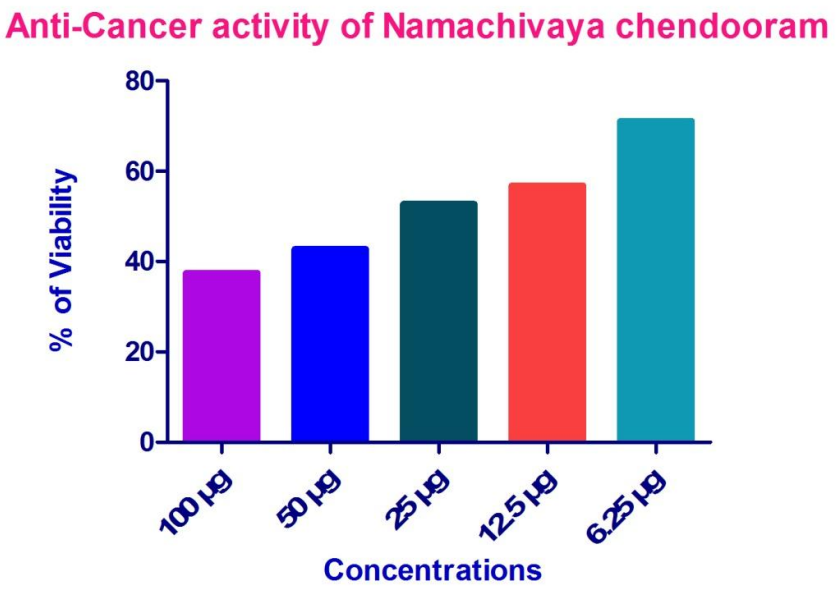
Sample Concentration (µg/ml)	Average OD at 540nm	Percentage Viability
Control	**0.9808**	
6.25	0.6979	71.1562
12.5	0.5587	56.9637
25	0.5180	52.8140
50	0.4201	42.8324
100	0.3687	37.5918

LD50 values – 46.29 µg/ml (ED50plus software V 1.0)

Cytotoxicity Assay by MTT

MTT colorimetric method, also known, is a method for detecting cell survival and growth methods. This assay is based on the metabolic reduction of 3- (4,5-dimethylthiazol-2-yl) -2,5-difeniltetrazol (MTT) by mitochondrial enzyme succinate dehydrogenase in a colored compound blue (formazan), allowing to determine the functionality of the mitochondrial treated cells. This method has been widely used to measure survival and cell proliferation. The amount of living cells is proportional to the amount of formazan produced. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination.

Namachivaya Chendooram at different doses (6.25-100 µg in100 µl of 5% MEM) was administered for 24 hrs. It was found that the number of cells decreases as the dose increases and at approximately 50 µg/ml dose of extract, 50% of the cells (HeLa cells) were less as compared to normal control as shown in figure no(9). The percentage of cells viability was determined by calculating the O.D of treated against thecontrol. Reading optical density (OD) is performed in a spectrophotometer at a wavelength of 540 nm.Comparison values are made on a basis of 50% inhibition of growth (IC_{50}) in treated cells with specific agents.Results are tabulated in Table (18) and graphically represented in Graph(1).



Graph-1

Graph-1 shows the drug dose and % of Inhibition of HeLa cells after the *Namachivaya Chendooram* extract treatment. It can be observed by the result of MTT assay that the IC dose of *Namachivaya Chendooram* is 50µg/ml. As the dose increases the HeLa cell viability decreases. It was found that the % growth inhibition increasing with increasing concentration of *Namachivaya Chendooram* steadily up to 6.25 µg/ml on *HeLa* cell line (Table (18) and Graph(1) and thatIC value on *HeLa* cell line was 50 and R value was 0.9808.

Analysis of Membrane Morphological Characteristics by Haematoxylin /Eosin (H/E) Staining

Morphological changes such as changes to the cell membrane, loss of membrane asymmetry and cell shrinkage, are the early stage of apoptosis was analyzed by H/E staining. The IC dose (50µg/ml) treated cancer cells show features of apoptosis whereas treated with same amount of dose, to normal treated cells appeared without any significant changes.

Since the discovery of the cisplatin antitumor activity, great efforts have focused on the rational design of metal-based anticancer agents that can be potentially used in cancer chemotherapy. Over the last four decades, a large number of metal complexes have been extensively investigated and evaluated *in vitro* and *in vivo*.
The key focuses of these studies lie in finding novel metal complexes which could potentially overcome the hurdles of current clinical drugs including toxicity, resistance and other pharmacological deficiencies.

Metals and metal compounds have been used in medicine for several thousands of years. The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance. Monographs and major reviews, as well as dedicated volumes, testify to the growing importance of the discipline [93][94].

Relevant reviews are to be found throughout annual series, for example Metal Ions in Biological Systems [95] [95a].

The field of inorganic chemistry in medicine may usefully be divided into two main categories: firstly, ligands as drugs which target metal ions in some form, whether free or protein-bound; and secondly, metal-based drugs and imaging agents where the central metal ion is usually the key feature of the mechanism of action [96].

Arsenic has been used therapeutically for more than 2,000 years and was used in the 1930s for treatment of chronic myeloid leukemia until supplanted by newer chemotherapies [97].

Side effects are cardiotoxicity, skin rashes, and hyperglycemia [98].

Oncologists and scientists engaged in the research of cancer treatments should conduct a comprehensive study on the efficacy of Mercury which is being used as an anti-cancer drug in the age old Siddha system. Three years of research has shown that metal (Mercury, arsenic and copper) based Siddha drug is a safe alternative for cisplatin therapy or arsenic trioxide in selected cases of cancer treatments wherein the patients cannot bear the adverse effects. He found that mice treated with Siddha drugs showed better health than what did in cisplatin therapy in terms of appetite, haemoglobin, red blood cells and white blood cells [99].

ANTI-TUMOUR ACTIVITY

Discussion

- To test the effect of on the growth kinetics, SiHa cells were treated with different concentrations of NMC:0,10,20,40 and 80µg/ml and were grown for 24,48 and 72h. At the end of each treatment, the cells were stained with trypan blue, and the viable cells that excluded the dye were counted.
- It was observed that there was a dose-dependent decrease in the growth kinetics of tNMC- treated cells compared to the untreated control cells (Fig.10A). Moreover, it was found that at around 80 µg/ml concentration of NMC treatment, there was a significant decrease (~2-fold) in the growth kinetics compared to that observed in the untreated control cells ($p \leq 0.05$ for 24h;$p \leq 0.001$ for 48h and 72h).

- This was further confirmed by colony forming assay wherein at a lower seeding density, cells were treated with different concentrations of NMC for one week. At 80 µg/ml concentration of NMC, the cells exhibited relatively lesser colonies compared to the control cells (Fig.10B).

- Consistent with the slow growth rate, it was observed that NMC extract induced a dose-dependent decrease in the number of soft agar colonies. Interestingly, at 80 µg/ml NMC treatment, the number of soft agar colonies was reduced by ~3-fold ($p \leq 0.001$) compared to the untreated control cells (Fig10C.).

- All these data indicated that NMC altered the growth kinetics of SiHa cells in a significant manner that could be a positive indicator for testing its anti-tumor activity in cervical cancer cells.

To further elucidate the anti-cancer mechanism of *NMC* in cervical cancer cells, we performed apoptosis studies. After treating the cells with different doses of *NMC*, the percent apoptotic cells were assessed by Annexin V-FITC and propidium iodide staining, followed by flow cytometric analysis(Fig.11). It was observed that at concentrations of 40 and 80µg/ml *NMC*, there was a significant increase in the percentage of cells undergoing apoptosis. Interestingly at 80µg/ml *NMC*

concentration, there was ~2.6-fold($p \leq 0.001$) increase in the population of cells undergoing apoptosis compared to the untreated control cells.

Discussion

- We found that the *NMC* extract significantly affected the growth rate of SiHa cells in a dose – dependent manner. This data was further supported by results from colony formation and soft agar assays, which demonstrated statistically significant reduction in the number of colonies in *NMC* treated cells compared to the untreated control cells. Thus, *NMC* could be proposed to be a promising candidate for restricting the growth of cervical cancer cells.

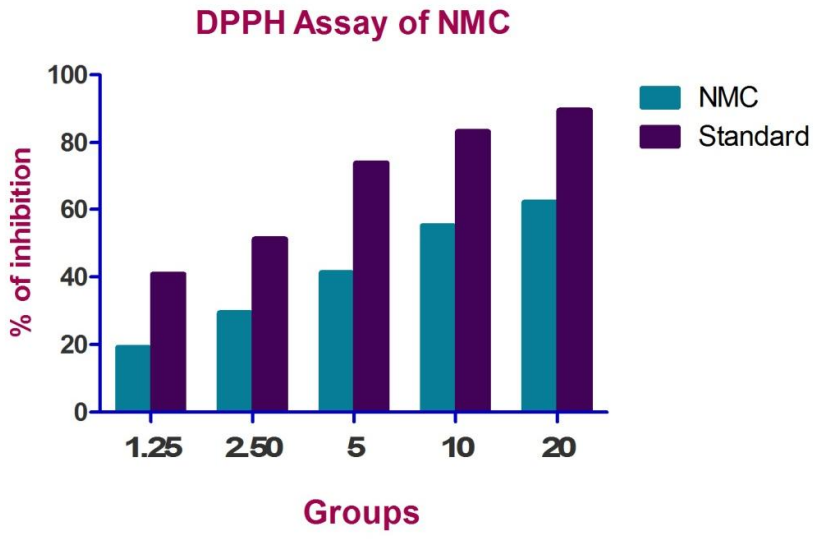
- Apoptosis plays a key role in the regulation of normal tissue homeostasis and participates in the elimination of abnormal cells. Most of the antitumor drugs kill the cancer cells by stimulating the apoptotic pathway [100]. To test whether *NMC* could induce apoptosis in cervical cancer cell line SiHa, we carried out apoptosis studies. At an effective *NMC* concentration of 80µg/ml, a significant proportion of cells were observed to undergo apoptosis compared to the control cells. To further elucidate the mechanism of apoptosis, we tested whether NMC could modulate calcium flux as the latter is known to be one of the major mediators of apoptosis.

Anti-oxidant Activity

DPPH Assay of *NAMACHIVAYA CHENDOORAM*
Table-19

Concentration (µg/µl)*	**Absorbance**		**Percentage of inhibition**	
Namachivaya Chendooram	Drug	Standard	Drug	Standard
Control	0.9846	0.341	-	-
1.25	0.9754	0.299	19.17*	40.89
2.50	0.9636	0.232	29.45	51.25
5.00	0.8849	0.114	41.32	74.07
10	0.7647	0.092	55.23	83.33
20	0.6432	0.054	62.21**	89.62

***µg/ml: microgram per milliliter. Drug: NMC(1.25-20µg/µl). Standard: Ascorbic acid(10mg/mlDMSO)*



Graph-3

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *Namachivaya Chendooram* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colorless stable molecule 1, 1 diphenyl-2-picryl hydrazyl is formed and as a result to which the absorbance at 517 nm of the solution is decreased. In the present study the *Namachivaya Chendooram* extract was analyzed was able to decolorize DPPH and the free radical scavenging activity was expressed as the percentage decrease in absorbance.

(10mg/mlDMSO) have a concentration-dependent anti-radical activity which was tabulated in Table No.19.

A maximum of 62.21% and 89.62% anti-radical effects are exercised by Namachivaya Chendooram and standard drug ascorbic acid at concentrations of 20 µg / ml respectively. Minimum percentage of inhibition 19.17% and 40.89% anti-radical effects are manifested by *Namachivaya Chendooram* and standard drug ascorbic acid at concentrations at 1.25µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and *Namachivaya Chendooram* extract. But the *Namachivaya Chendooram* extract has lower DPPH scavenging activity than that of standard. From the present study, it was concluded that the *Namachivaya Chendooram* extract has good anti-oxidant activity at higher concentrations.

It is known that oxidative stress induced cell damage not only through damage to proteins, lipids and DNA. It may also alter signaling pathways redox sensitive to changes involved in the response of apoptosis. The antioxidants are currently the subject of many studies because, in addition to some interest in the preservation of comestibles, they could be useful in the prophylaxis and treatment of diseases in which oxidative stress is implicated. Many studies realized on natural products have proven that they are especially phenolic compounds who are responsible for their antioxidant activity.

Several studies have shown the link between the traditional drug formulations rich in antioxidants and the incidence certain diseases such as **cancer**, heart disease, diabetes and other diseases related to aging. Phenolic compounds could prevent cancer by the action antioxidant and / or the modulation of several functions of proteins. Phenolic compounds can prevent carcinogenesis by affecting the molecular events in the triggering, promotion and progression stages.

Here, the reactive oxygen species (ROS) may be the triggers apoptotic process. In recent years they have been described numerous properties of these compounds such as the ability to inhibit cell cycle, proliferation cellular and oxidative stress, and induce detoxification enzymes, apoptosis, and stimulate the immune system. It is therefore hypothesized that *Namachivaya Chendooram* of its antioxidant power could “to repair" Cancer cells.

6. CONCLUSION

Cervical cancer is the second most common malignancy found among women worldwide and some are highly resistant to radiotherapy. The other chemotherapy drugs also deliver intolerable side effects which are worse than the disease. This paved way for a novel anticancer drug which cures cervical cancer in a non invasive way.

The intention of this study is to provide a solution for the above need. For a non violent anticancer drug to cervical cancer, ***Namachivaya Chendooram*** was chosen from the Siddha literature as a trial drug "**Prana Raksha Mirtha Sindhu Vaithya Ratna Sangaragam**" written by T.R. Mahadeva Pandidar which was categorized by the department of AYUSH as a classical text. Throughout the study, the safety and efficacy were tested thoroughly.

The procedure for drug preparation and its techniques for standardization revealed GMP. The trial drug *NMC* has satisfied all parameters of testing protocol for Chendooram which was assigned by AYUSH. It showed the accurate production and potency of *Namachivaya Chendooram*

Physico-chemical analysis revealed better bio-availability and richness of its mineral content. Favouring this study were the presence of inorganic matters which were found through experiments for analyzing acid and basic radicals.

Various instrumental analysis of *Namachivaya Chendooram* such as FT-IR spectroscopy, X-ray fluorescence spectrometer and scanning electron microscope demonstrated its chemical constituents, functional groups and particle size to support its indication to counter cervical cancer.

The anti-microbial activity of trial drug was also considered for its potential.

Under OECD guidelines, the acute and 28 days repeated oral toxicity studies proved the safety of *Namachivaya Chendooram* at particular dose level. It is very useful in therapeutic dose determination.

The pharmacological activities are justified by anticancer effect on HeLa cell lines, anti-tumour effect on SIHA cell lines and quantitative measurement of antioxidants by DPPH assay.

Factors like safety, efficacy, long self like, bio-availability, presence of significant elements, anions and cations and minerals favouring the activity justifies the main perspective of this study.

NMC's anti-cancer effect could be validated scientifically. Due to its Non-toxic anti-cancer effect, it would benefit the health community and the world.

FUTURE SCOPE

Trial drug for the study ***NAMACHIVAYA CHENDOORAM*** was taken from the classic Siddha Literature **Prana Rakshamirtha Sindhu Vaithya Ratna Sangaragam** Written by **T.R. Mahadeva Pandidar** Its validation for its Anti-cancer nature was completed at preliminary level. The result enhanced and assured its Anti-cancer property against cervical cancer.More specific experiments on animal models and also clinical trials are required to understand the exact molecular mechanisms of action. So it could be used worldwide in treatment of cervical cancer and satisfy the safe and painless anti-neo plastic treatment.

7. SUMMARY

Trial drug ***Namachivaya Chendooram*** was selected from the classic literature "**Prana Rakshamirtha Sindhu Vaithya Ratna Sangaragam**" written by T.R.Mahadeva Pandidar for its anti-cancer, anti-tumour, anti-oxidant activities.

The dissertation started with an introduction explaining about the Siddha concept, prevalence of cervical cancer and role of the test drug in treating cancer cervix.

- Review of literature in various categories was carried out .It was elaborated under Gunapadam and Modern aspect of ingredients, Siddha and modern aspect of that disease, pharmaceutical aspect and pharmacological aspect in both Siddha and modern.
- All the ingredients were identified and authenticated by experts.
- The compound was prepared properly by given procedure in an appropriate situation.
- The end product underwent standardization parameters in Siddha.
- The drug was subjected to analysis such as physiochemical, biochemical and instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug.
- The sample was also analysed for anti-microbial activity to ensure its accuracy.
- For the study protocol, required animals were approved by the IAEC under CPCSEA.
- Toxicological study was made according to OECD guidelines comprising both acute and repeated oral dose 28days toxicity studies in wistar albino rats. It showed the safety of the drug which attributes its utility in long time administration.
- Pharmacological studies were completed. It revealed the anti-cancer, anti-tumor and anti-oxidant activities of *Namachivaya Chendooram*.
- Results and discussion gives the essential validations to prove the potency of the drug.
- Conclusion gives a Compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

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